The photosynthetic apparatus of *Rhodobacter sphaeroides*

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Functional and ultrastructural studies have indicated that the components of the photosynthetic apparatus of *Rhodobacter sphaeroides* are highly organized. This organization favors rapid electron transfer that is unimpeded by reactant diffusion. The light-harvesting complexes only partially surround the photochemical reaction center, which ensures an efficient shuttling of quinones between the photochemical reaction center and the bc_1 complex.

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The predominantly green color of the biosphere attests to the essential role of photosynthesis on Earth. By this process, plants convert light energy into chemical energy to reduce carbon dioxide to organic matter such as carbohydrates. This capability is, however, not limited to plants. Certain bacteria are also able to perform this energy conversion for their growth and development. The molecular machinery involved in the initial steps is very similar in plant and bacterial photosynthesis, and purple bacteria are the model bacterial system for this process. One of the most often studied species is *Rhodobacter sphaeroides*. This Gram-negative bacterium of the Proteobacteria group is metabolically highly diverse. *R. sphaeroides* can grow photosynthetically or heterotrophically via aerobic or anaerobic respiration[1]. Heterotrophic growth allows the isolation of mutants impaired in photosynthesis.

Over the past 40 years, a combination of biophysical, biochemical and genetic approaches has provided us with a detailed description of the functions of the different proteins involved in photosynthesis. In particular, the cascade of molecular events that follows the absorption of a photon has been determined in fine detail using time-resolved spectrophotometry.

Photo-induced cyclic electron transfer

Most of the photosynthetic machinery of *R. sphaeroides* is located within the invaginated inner membrane that forms during photosynthetic or dark, semi-aerobic growth[2]. The photosynthetic apparatus is composed of three multimeric transmembrane protein complexes: the antenna or light-harvesting complexes (LHC), the reaction center (RC) and the cytochrome (cyt) bc_1 complex. The role of the LHCs, which non-covalently bind carotenoid and bacteriochlorophyll molecules, is to collect incident light. Like most purple bacteria, *R. sphaeroides* has two types of LHCs, termed LH1 and LH2. The amount of LH2 is modulated by several factors, such as light intensity and oxygen partial pressure, whereas the LH1 complement is synthesized in fixed stoichiometric amounts with the RC, forming the RC–LH1 complexes[3]. The large amount of antenna pigments with respect to the RC (up to 100 bacteriochlorophyll molecules are present per RC) increases the cross section available for light capture.

When a photon is absorbed by the LHC, the excitation reaches the RC (where charge separation occurs) in less than 100 picoseconds (ps). At the RC, an electron is transferred from the excited primary donor, a bacteriochlorophyll dimer, to a molecule of bacteriopheophytin in 2–3 ps, and, subsequently, to the primary quinone (Q_A) in ~200 ps. These fast electron transfers stabilize the separated charges and ensure a quantum yield close to 1 (i.e. for almost every photon absorbed by the RC, one electron is transferred). During the few tens of microseconds following light excitation, the photooxidized primary electron donor is reduced by the periplasmic protein cyt c_2; on the acceptor side, the electron is transferred from Q_A^- to a second ubiquinone molecule (Q_B), forming an RC-bound semiquinone (Q_B^-). Q_B^- undergoes a second reduction step during the next photochemical turnover of the RC. This doubly reduced Q_B picks up two protons from the cytoplasmic space and is then released from the RC, joining the quinone pool. The electron-transfer cycle is completed by the oxidation of the quinol by cyt c_2 via the [Fe_2S_2] cluster of the Rieske protein, a reaction catalysed by the bc_1 complex, and the release of two protons into the periplasmic space (Fig. 1). Cyclic electron transfer is thus coupled to the translocation of protons from the cytoplasm to the periplasm and creates a proton-motive force that drives ATP synthesis and NAD^+ reduction.

Another membrane protein, the PufX polypeptide, is essential for anaerobic photosynthetic growth[4]. This small polypeptide is closely associated with the RC–LH1 core complexes in a 1:1 ratio[5]. It is encoded by the *pufX* gene, localized in the *puf* (photosynthetic unit formation) operon downstream of the genes encoding the LH1 subunits and the L and M subunits of

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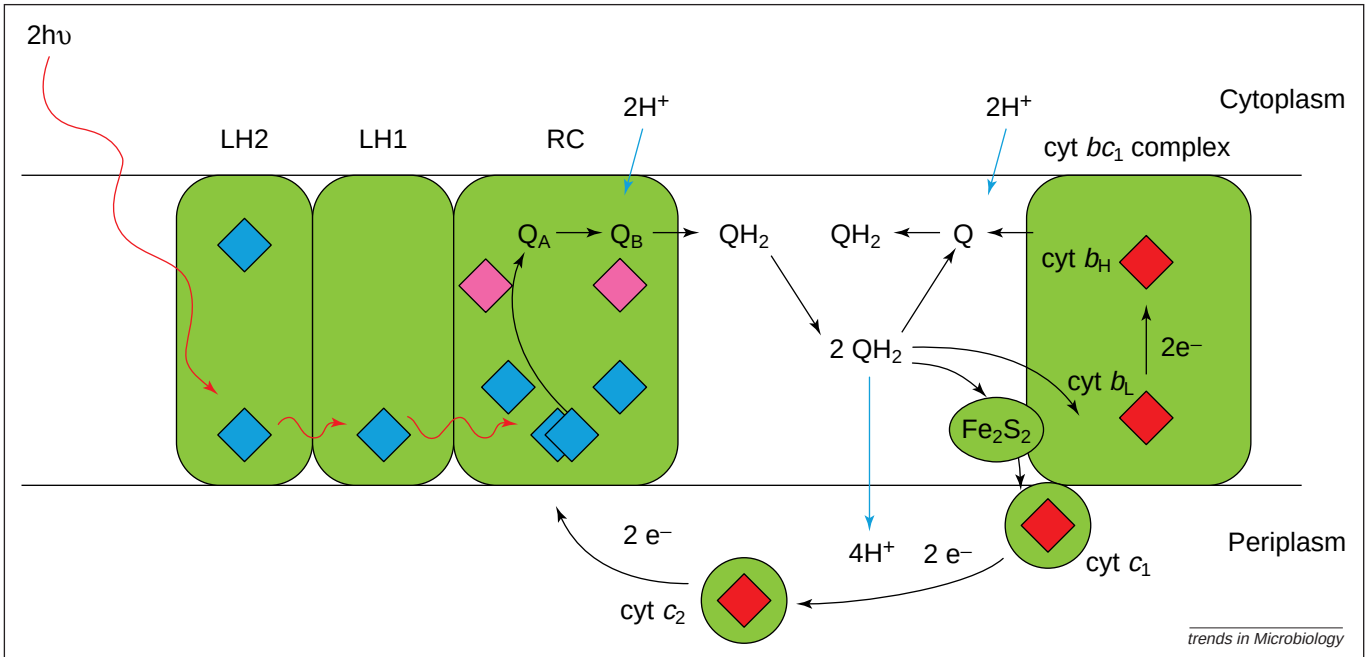

Fig. 1. Schematic representation of the photoinduced cyclic electron-transfer chain in *Rhodobacter sphaeroides*. Bacteriochlorophyll molecules are represented by blue diamonds and bacteriopheophytin molecules by pink diamonds. Hemes are denoted by red diamonds. The red arrows represent excitation transfer and the black and blue arrows correspond to electron and proton transfer, respectively. The stoichiometry of electrons and protons corresponds to the absorption of two photons (hυ) by the reaction center (RC). After absorption of a photon by the light-harvesting complexes (LH1 and LH2), the excitation reaches the reaction center, where charge separation occurs. An electron is transferred from the excited primary donor of the RC, a bacteriochlorophyll dimer, to the secondary acceptor Q_B via a molecule of bacteriopheophytin and the primary quinone, Q_A. After a second turnover, the doubly reduced Q_B picks up two protons from the cytoplasmic space. The quinol (QH_2) formed is oxidized by cyt c_2, a reaction catalysed by the bc_1 complex, which releases two protons to the periplasmic space. The cyclic electron transfer is completed by the reduction of the photooxidized primary electron donor by cyt c_2.

the RC (Ref. 6). Deletion of *pufX* impairs turnover of the RC by strongly retarding the quinone exchange between this complex and the bc_1 complex[7].

Structure–function issues in the photosynthetic apparatus

A major breakthrough in the elucidation of the molecular events of photosynthesis was the resolution of the structure of the RC of *Blastochloris* (formerly *Rhodopseudomonas*) *viridis* by X-ray diffraction[8]. This pioneering work marked a new era in membrane-protein biochemistry. The subsequent description of the structure of the RCs of *R. sphaeroides*[9] illustrated the high structural homology between RCs from different species (Fig. 2). More recently, the 3-D structures of LH2s isolated from different photosynthetic bacteria[10,11] and of the mitochondrial bc_1 complex have been resolved[12] (Fig. 2). Despite the abundance of information available on the individual complexes, the characterization of their organization and assembly on a broader scale in the native membrane remains a challenge.

The efficient transfer of excitation (>90%) between the large number of LHC pigments and the RCs necessitates close proximity of the different chromophores (≪50 Å) and, therefore, a specific organization of these complexes in the membrane. By contrast, it is generally believed that no particular arrangement is required for electron transfer between the RCs and the bc_1 complexes. However, analysis of the lateral distribution of membrane complexes by freeze-fracture electron microscopy indicates a unique spatial arrangement of the particles relative to one another, suggesting the existence of well-defined structural entities[13]. The connection between these complexes is assumed to be mediated by random collisions between free, diffusive electron carriers such as quinone molecules in the membrane and cyt c_2 in the periplasmic space (Fig. 1). However, here we will summarize the biochemical, functional and structural data that indicate a high degree of organization of the electron-transfer chain in the native membrane of *R. sphaeroides*.

A ring structure of LHCs around the RC

Gentle detergent treatment of the inner membrane of photosynthetic bacteria can specifically extract LH2 complexes and RC–LH1 complexes. A clear picture of the close association of LH1 with the RC was provided by Miller's early low-resolution electron microscopic analyses of native membranes of *B. viridis*[14]. The RC–LH1 complexes of this bacterium, which has no LH2 and a low number of bc_1 complexes, naturally form 2-D crystals. This work revealed that a ring of LH1 molecules surrounds a single RC. This organization of the complexes has been confirmed for several other species of photosynthetic bacteria[15–17]. In particular, Walz and colleagues[17] have recently obtained RC–LH1 crystals from *R. sphaeroides* of sufficient quality to show that the RC is surrounded by 15–17

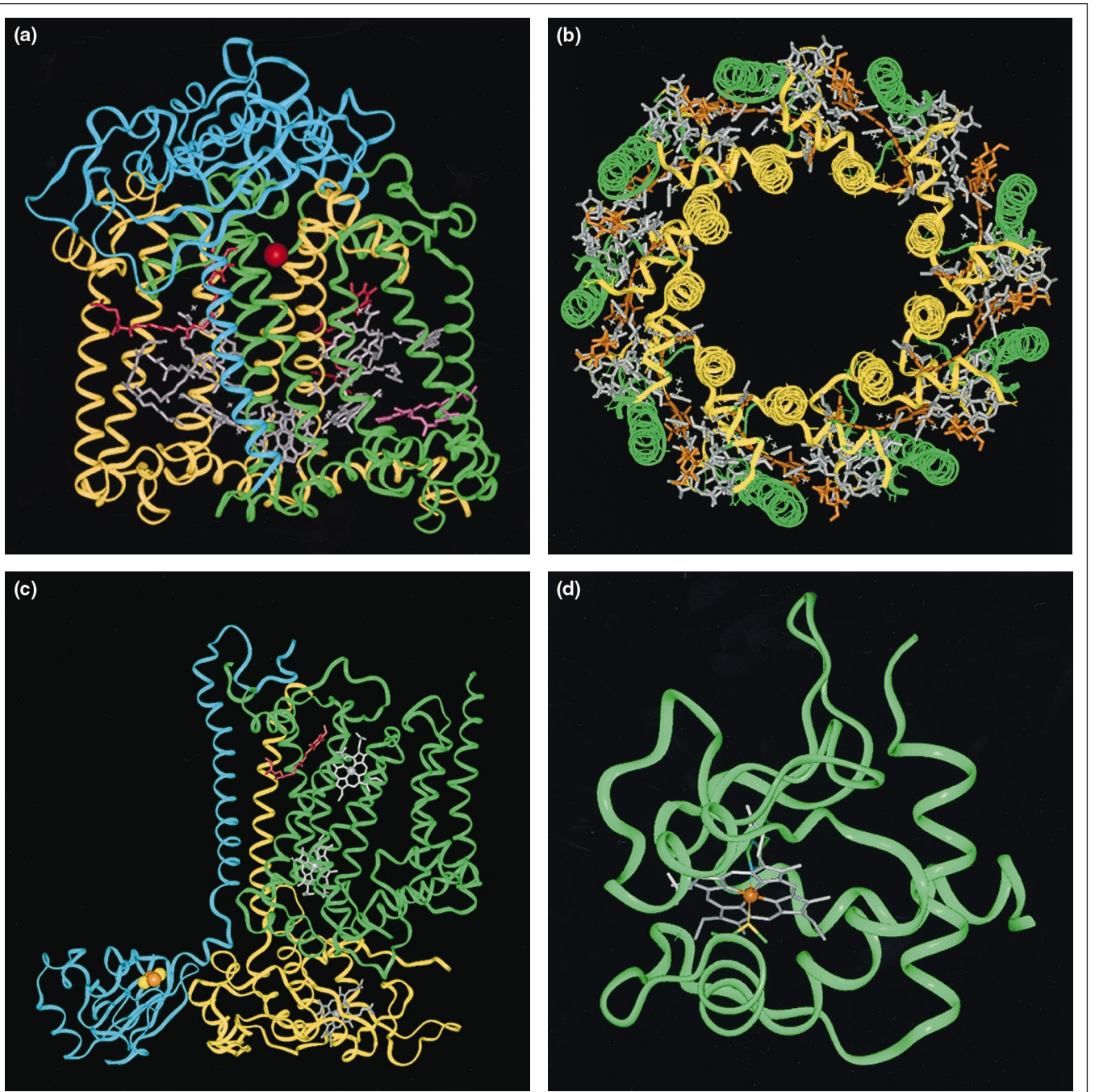

Fig. 2. 3-D structures of the different components of the photosynthetic chain. **(a)** Reaction center (RC) of *Rhodobacter sphaeroides*[38]. The L, M and H subunits are shown as yellow, green and blue ribbons, respectively. The bacteriochlorophyll and bacteriopheophytin molecules are in grey. The primary and secondary ubiquinone molecules are in red. The carotenoid molecule is pink. The reaction center presents a pseudo-C2 symmetry around an axis normal to the membrane plane going through the iron atom (red sphere) located between the two ubiquinone molecules. Only the L-branch is photochemically active. The view is parallel to the membrane plane as in Fig. 1. **(b)** Light-harvesting complex 2 (LH2) from *Rhodopseudomonas acidophila*[10]. The α and β subunits are shown as yellow and green ribbons, respectively. The bacteriochlorophyll molecules are in grey and the carotenoid molecules in orange. This view is from the periplasmic face of the membrane. **(c)** Catalytic subunits of the mitochondrial bc_1 complex[12]. The Rieske subunit is shown in blue. The Fe and S atoms of the $[Fe_2S_2]$ cluster are represented by orange and yellow spheres, respectively. The cytochrome b subunit is shown in green and the cytochrome c_1 subunit in yellow. Hemes are colored in grey and the ubiquinone molecule in red. **(d)** Cytochrome c_2 from *Rhodobacter sphaeroides*[39] depicted as a green ribbon with the heme represented in grey.

LH1 subunits. The closed structure of LH1 complexes, combined with the tight coupling of the bacteriochlorophyll molecules, ensures a rapid delocalization of the excited state and the possibility for energy to be transferred from any point of the ring. However, the packed organization of this structure raises the question of how the quinone–quinol transfer takes place between the RC acceptor side and the bc_1 complex during photoinduced electron transfer (Fig. 1).

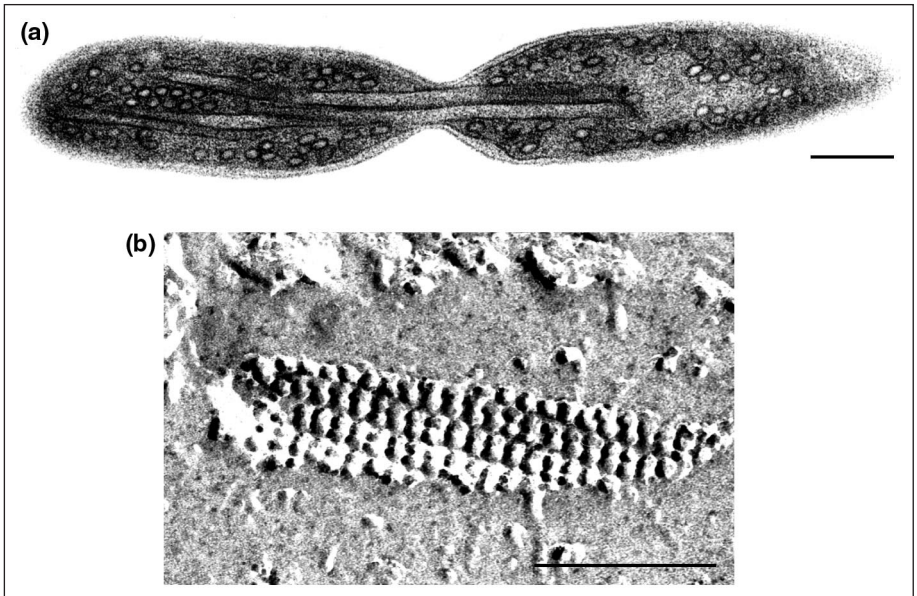

Fig. 3. (a) Thin-section electron micrograph of *Rhodobacter sphaeroides* cells grown under photosynthetic conditions in the presence of nitrate. Under these growth conditions, the intracytoplasmic membrane presents long tubular structures (100 nm in diameter and several hundreds of nm in length) in addition to the chromatophores (spheres of 55–70 nm in diameter). The formation of the tubes is due to the lack of light-harvesting complex 2 (LH2) complexes in this part of the intracytoplasmic membrane. These tubes contain all the membrane components of the photosynthetic apparatus (i.e. reaction center, bc_1 complex and LH1 complexes). Scale bar = 200 nm. (Micrograph courtesy of J. Jappé.) **(b)** Freeze-etching electron micrograph of the tubes induced by the presence of nitrate showing a well ordered arrangement of dimeric particles of the size of RC–LH1 complexes (110 Å). Scale bar = 100 nm. (Micrograph courtesy of Dr J. Olive.)

Evidence for a supercomplex arrangement

A series of thermodynamic and kinetic studies has provided new insight into the organization of the components of the photosynthetic electron chain. A key observation was that the 'apparent equilibrium constants' between the different reactants (RC, bc_1 complex and cyt c_2), measured during the photooxidation of these carriers, were much lower than those deduced from their mid-point potentials determined at equilibrium (in the presence of redox mediators) in the dark[18]. This behavior can be explained as follows. In the photooxidation experiment, a rapid equilibrium is achieved at a local level, within specific domains or supercomplexes containing a small number of electron carriers, whereas equilibration at a macrolevel between these domains is a much slower process[19]. Data analysis suggests that each supercomplex contains two RCs closely associated with one bc_1 complex and one cyt c_2 (Ref. 18), in agreement with the overall stoichiometry of these complexes in the chromatophore membrane. In other words, the close association of the RCs and the bc_1 complex results in the trapping of one molecule of cyt c_2 per RC dimer, hindering its diffusion into the periplasmic space.

Crofts *et al.*[20] have, however, proposed an alternative interpretation of the observed kinetic and thermodynamic anomalies. They assume free diffusion of the electron transfer components in the aqueous phase, but heterogeneity in the stoichiometry of membranes complexes and cyt c_2 in the chromatophore population.

Another line of argument in favor of a supramolecular organization of components of the photosynthetic chain comes from the observation that the addition of a subsaturating concentration of myxothiazol, a specific inhibitor of the bc_1 complex, decreases the number of active bc_1 complexes but does not affect the rate of electron transfer for the uninhibited complexes[21]. Moreover, the cyt c_2 connected with the inhibited bc_1 complexes remains photooxidized because it cannot interact with the uninhibited chains. This behavior implies that each photosynthetic chain acts as an isolated entity. Interestingly, Fernandez-Velasco and Crofts[22] observed different behavior using intact cells or isolated chromatophores. In isolated chromatophores, cyt c_2 appears to freely diffuse and interact with all the bc_1 complexes present in the vesicles. A possible explanation for this discrepancy could be the loss of supercomplex association during the preparation of the isolated chromatophores.

A more convincing indication of a supramolecular organization of the photosynthetic electron carriers is that the complete photoinduced electron-transfer cycle occurs at −20°C in frozen medium[23]. Under these conditions, the overall rate is limited by the reaction between cyt c_2 and the photooxidized primary electron donor. The lateral movements of cyt c_2 between the RCs and the bc_1 complex are not rate-limiting, which argues for a necessary proximity of these reactants.

Supramolecular organization in native membranes

As well as the functional arguments, ultrastructural evidence of a supramolecular organization of the photosynthetic components has recently been obtained from freeze-fracture electron microscopy of tubular membranes from *R. sphaeroides* cells[24,25]. These tubular membranes (100 nm in diameter and several hundred nm in length) correspond to regions of the intracytoplasmic membrane that do not contain LH2 complexes. They are formed when the amount of LH2 complexes is decreased, either by gene deletion[26,27], growth under semi-aerobic conditions or the presence of nitrate[25] (Fig. 3). The freeze-fracture images reveal a well ordered arrangement of dimeric particles of ~110 Å in diameter (i.e. the size of RC–LH1 complexes) (Fig. 3). These tubular membranes contain all the membrane components of the photosynthetic apparatus, in the relative ratio of one bc_1 complex to two RCs and approximately 24 bacteriochlorophyll molecules per RC (Ref. 28). As a result of the well ordered arrangement of the particles in these membranes, electron micrographs of negatively

stained samples diffract up to 25 Å (Ref. 28). The calculated projection map of the upper face of these tubes is depicted in Fig. 4. The unit cell contains an elongated S-shaped supercomplex composed of two C-shaped structures with an external diameter of 112 Å, closely matching the dimeric arrangement in the freeze-fracture samples. The open sides of the C-shaped structure face each other and enclose a large protein mass. Although a definitive interpretation of this projection map cannot be given at present, it is assumed that each C-shape corresponds to LH1 complexes partially surrounding one RC.

A dimeric association of RCs is in agreement with the results obtained by Francia *et al.*[5]. After detergent solubilization of *R. sphaeroides* chromatophores, they found two membrane complexes corresponding to monomeric and dimeric RC–LH1 complexes, in addition to isolated LH1 and LH2 complexes. Electron micrographs show that the dimeric RC–LH1 complexes comprise two intertwined rings of LH1 containing two RCs (Ref. 5). It was also shown that the PufX polypeptide is strictly required for the isolation of the dimeric RC–LH1 complexes. As low concentrations of the PufX polypeptide inhibit the *in vitro* oligomerization of LH1 complexes[29], one possibility is that PufX plays an essential role both in the supramolecular arrangement of the photosynthetic apparatus and in the C-shaped structure of the LH1 complexes by restraining the formation of a closed ring. In this context, it is interesting that deletion of *pufX* induces a significant increase in the ratio of bacteriochlorophyll molecules to RCs, which might be related to the formation of a closed ring around the RC (Refs 30,31). The C-shaped geometry of the LH1 complexes would facilitate the diffusion of quinone molecules between the RCs and bc_1 complexes by creating a path between them. This is in contrast to other electron microscopic studies of monomeric purified RC–LH1 complexes, in which the RCs are surrounded by a closed LH1 ring. The principal difficulty in the interpretation of the projection map of Fig. 4 is the localization of the bc_1 complex present in these tubular membranes. The electron density localized outside the C-shaped structures is tentatively attributed to the bc_1 complex.

Fig. 4. Projection map at 20 Å resolution of a negatively stained native tubular membrane of *Rhodobacter sphaeroides*. Positive densities from the proteins are indicated as solid lines. The basic unit, 198 Å long and 112 Å wide contains an elongated S-shaped supercomplex composed of two C-shaped structures facing each other. Each C-shape might correspond to light-harvesting complex 1 (LH1) partially surrounding one reaction center (RC). The electron density localized outside these structures is tentatively attributed to the bc_1 complex. Adapted, with permission, from Ref. 28.

Supercomplexes: a common feature?

Are these supercomplexes a general feature of the electron-transfer components of photosynthetic bacteria? The answer is almost certainly no. Even in the case of *R. sphaeroides*, the photosynthetic chains localized in the non-invaginated part of the cytoplasmic membrane do not appear to be organized in supercomplexes and share cyt c_2 and bc_1 complexes with the respiratory chains[32]. However, the specific association of cyt c_2 with the RCs and the cyt bc_1 complex in the invaginated part of the membrane limits its interaction with other complexes present in the cytoplasmic membrane or the periplasm, such as cytochrome oxidase or the denitrification enzymes; this favors photosynthetic activity over respiration or denitrification. Other photosynthetic bacteria, such as *B. viridis* and *Rhodospirillum rubrum*, possess a large excess of RCs over bc_1 complexes; the formation of supercomplexes of the *R. sphaeroides* type is, therefore, stoichiometrically restricted. However, in the case of *R. rubrum*, only one molecule of cyt c_2 can bind to two RCs, showing that the dimeric organization of the RCs can be maintained in the absence of the bc_1 partner[33]. A dimeric association has also been observed *in vivo* for the RCs of photosystem II in green plants, which are highly homologous to the RCs of purple bacteria[34].

Are other bioenergetic chains organized in supercomplexes? According to the chemiosmotic hypothesis, no specific supramolecular organization of membrane complexes is required, provided their coupling is completed by liposoluble and hydrosoluble diffusive carriers. Nevertheless, organization of electron

Questions for future research

- Is the open ring a general feature of light-harvesting complex I (LHI) in photosynthetic bacteria?
- Are the quinone molecules confined to the supercomplex?
- What is the exact role of PufX?
- Are supercomplexes between photosystem II and b_6f complexes present in the thylakoid membrane?
- Are membrane complexes of other bioenergetic chains organized in supercomplexes?

carriers in supercomplexes allows a more efficient electron transfer, unimpeded by reactant diffusion; indeed, supramolecular associations have been observed for membrane complexes involved in the respiratory chain of a few bacteria[35,36]. A similar situation is encountered for soluble enzymes involved in cellular metabolism. In different enzymatic pathways, intermediates are transferred from one enzyme to another without complete equilibration with the surrounding medium[37]. This 'metabolic channelling' also implies the existence of specific organization in the form of multienzyme complexes. Formation and dissociation of supercomplexes could be a general and powerful way that bacteria have developed to adapt their bioenergetic processes efficiently to the available energy source.

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