Arch Microbiol (1985) 142:333–339

Archives of
Microbiology

The *Rhodopseudomonas viridis* photosynthetic membrane: arrangement in situ

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Abstract. The organization of photosynthetic membranes in the cytoplasm of the photosynthetic bacterium *Rh. viridis* has been examined by several techniques for electron microscopy. Thin sections of membrane stacks show that the regular lattice of membrane subunits reported in other studies can be observed in thin section. Tilting of sections in the electron microscope shows that the regular lattices of several membranes overlap in a way that suggests they are in register with each other. This observation can be confirmed by freeze-fracture images in which a regular arrangement of membrane lattices can be observed, each perfectly aligned.

Analysis of the spacings of membrane pairs shows that the photosynthetic membranes of *Rh. viridis* are very closely apposed. The mean diameter of two membranes is 160A, and the average space between two such membranes is only 42A. When a recently developed atomic level model of *Rh. viridis* reaction center is superimposed against these spacings, each reaction center extends from the surface of its respective membrane far enough to make contact with an apposing membrane. The limited free space between membranes and regular alignment of lattices has a number of implications for how this membrane is organized to carry out the process of energy transfer.

Key words: Photosynthesis – Membrane structure – Electron microscopy – Photosynthetic bacteria

Rhodopseudomonas viridis is a purple, non-sulfur photosynthetic bacterium. Like other bacteriochlorophyll b-containing bacteria (Engelhardt et al. 1983), *Rh. viridis* contains photosynthetic membranes which are organized in a regular hexagonal pattern. This regular pattern has allowed the basic substructure of the membrane to be analyzed by two dimensional (Miller 1979; Wehrli and Kubler 1980; Stark et al. 1984) and three dimensional (Miller 1982) image-processing techniques. More recently, Michel and associates have reported on a structure for the *Rh. viridis* photosynthetic reaction center, a component of the photosynthetic membrane, at atomic resolution (Michel 1983; Michel et al. 1984).

The rapid emergence of detailed structural information about the organization of major components of this energy-transducing membrane has prompted us to examine the arrangement of membranes within the bacterial cell. The basic organization of the *Rh. viridis* cytoplasm was first reported by Giesbrecht and Drews (1966). In this paper we report on a number of new observations concerning the organization of the photosynthetic membranes within the cell, and describe the implications of imposing the atomic level structural map of the reaction center on data obtained from electron microscopy of intact cells.

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Materials and methods

Cultures of *Rh. viridis* were grown from originals kindly supplied by Dr. John Olson. The organisms were cultured and prepared for electron microscopy as previously described Miller (1979). All micrographs were taken on a Philips 410LS electron microscope operated at 100 kV.

Results

Basic arrangement of the membranes

The photosynthetic membranes of *Rh. viridis* are lamellar in nature, and are generally found in tightly packed stacks, as shown in Fig. 1. Each cell seems to contain a single large stack of membranes, which appear to be appressed in a manner very similar to the grana stacks of higher plants. As shown in Fig. 1, each thylakoid membrane forms a closed sac. Membrane appression is only observed on the outer surface of each stack, and never on the inner surface, again very similar to higher plants. In the region of membrane contact, the area of highest electron density actually seems to be the central region between the two opposite membranes.

Several studies using the freeze-fracture and negative staining technique have described the crystalline substructure of the membrane (Engelhardt et al. 1983; Miller 1979; Wehrli and Kubler 1980; Stark et al. 1984; Miller 1982; Giesbrecht and Drews 1966). It has been difficult to show, however that the *Rh. viridis* membrane is crystalline in all regions of the thylakoid. Is it possible that some regions display the crystalline substructure which has been used for structural analysis, while other regions display a less ordered arrangement of components? We prepared stereo micrographs of a number of regions where the thylakoids were organized into small stacks of membrane. A typical stereo pair is shown in Fig. 2. In each case where stereo images were examined, a detailed crystalline substructure could be observed in the membranes. This arrangement was partic-

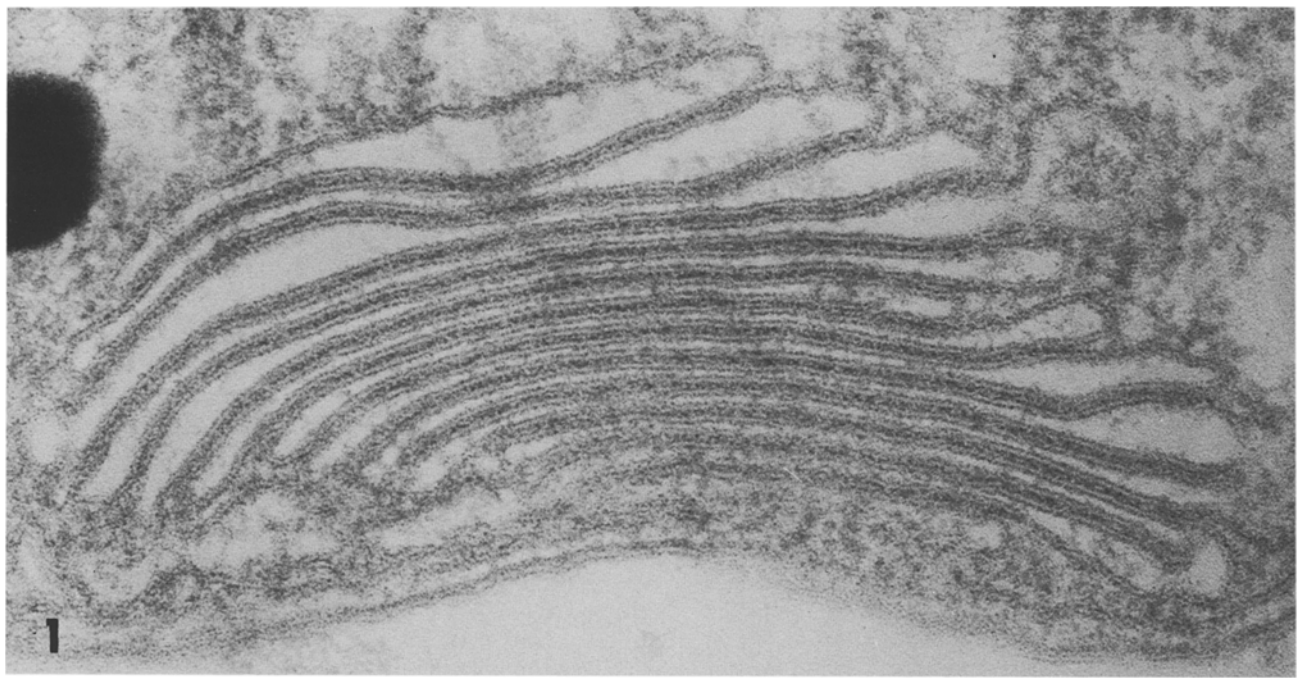

Fig. 1. Thin section of a stack of photosynthetic membranes found in the cytoplasm of *Rhodopseudomonas viridis (Rh. viridis)*. Each membrane is a closed sac. The membranes adhere at their outer surfaces to form such stacks, and are separated from each other by a zone of high electron density at the point of apparent contact. Magnification: 120,000

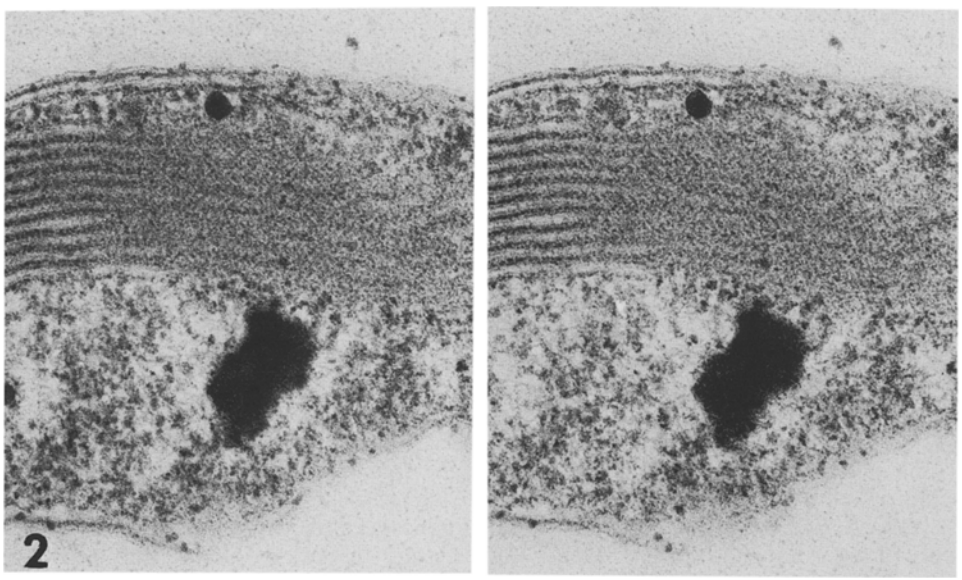

Fig. 2
Stereo pair of a stack of membranes visible in a thin of a *Rh. viridis* cell. The plane of sectioning has cut through the stack at a right angle towards the left side of each image, while the orientation of the membranes is more oblique towards the right hand side. The lattice-like arrangement of subunits within the membrane is most apparent in the oblique region. The ability to visualize such lattice-like regions wherever membranes have been sectioned obliquely suggests that all of the membranes of a stack contain the lattice substructure. Magnification: 58,000

ularly striking where the membranes were slightly oblique to the plane of sectioning, as seen at one end of the stack in Fig. 2.

Do all the membranes of a stack share a common orientation?

The regular arrangement of subunits which could be observed in many stereo micrographs suggested to us that each of the several membranes of a stack were arranged in patterns which had a common orientation. Many cells display large stacks of membranes (see Fig. 3) which may contain as many as 40 individual thylakoid membranes. In Fig. 3, regular periodicities are observed across several membranes in a stack, especially where the plane of the section is oblique. Such features suggest that the regular lattices found in each membrane of the stack share a common direction, because random orientation of each membrane in a stack could not produce such periodicities.

We tested the ability of membrane stacks to produce such regular features by tilting sections in a direction parallel to the planes of the membrane themselves. One such test is shown in Fig. 4, where a stack of thylakoid membranes

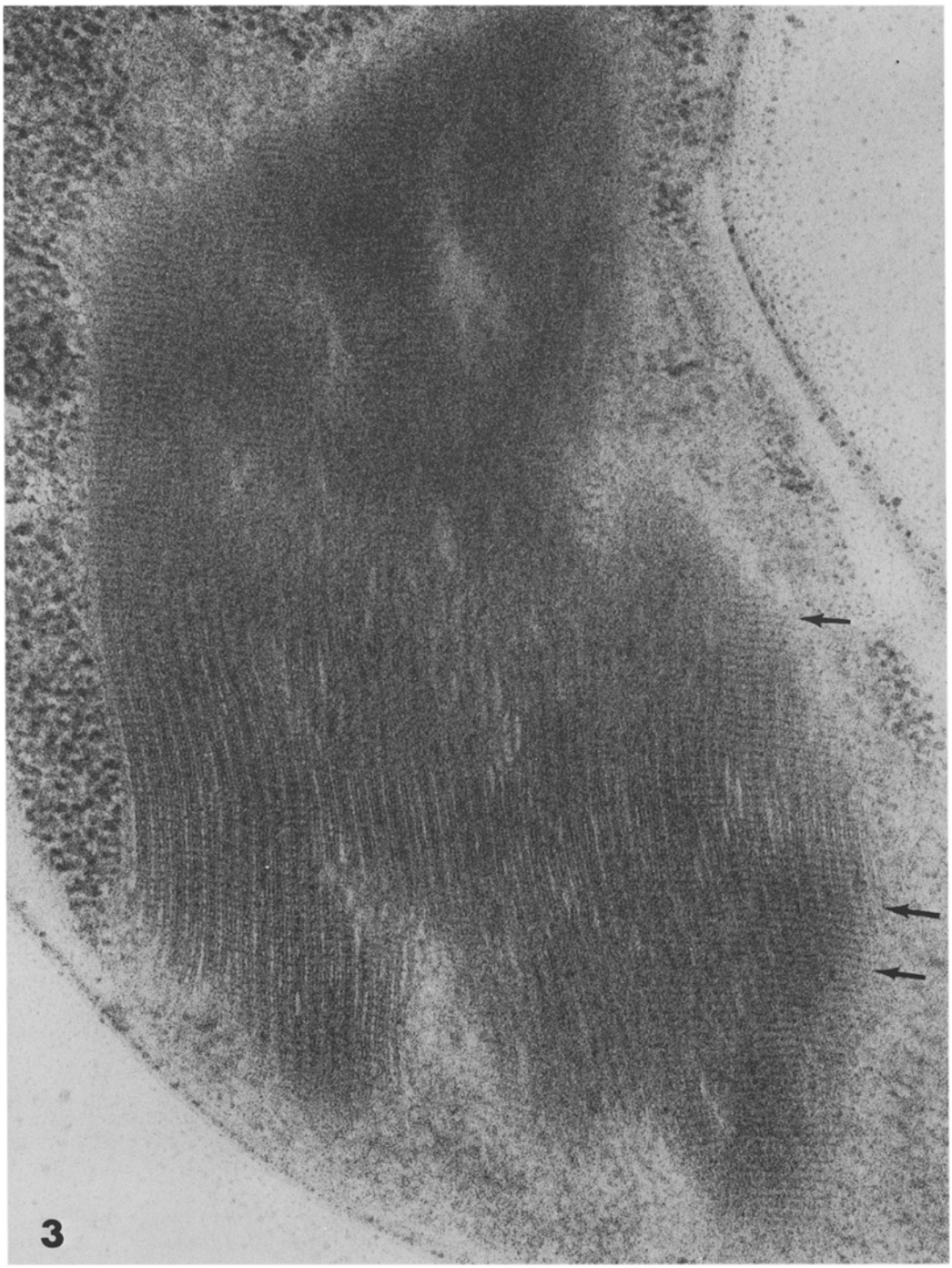

Fig. 3. A very large stack of membranes, including more than 50 individual membranes. Despite the large number of membranes, the regular lattice-like features of each one is visible in thin section. Regular striations of high density (*arrows*) are visible at intervals of approximately 13 nm, corresponding to center-to-center distance between subunits within the membrane lattice. Magnification: 115,000

has been tilted through three different angles. Figure 4a illustrates a 24° tilt in which the membranes are nearly parallel to the direction of observation. As the section is tilted back towards normal orientation (Fig. 4b: 4°) and then beyond (in Fig. 4c: −27°), the profiles of individual membranes become blurred, and regular lattice spacings at 130A intervals appear (see arrows).

As shown in Fig. 4, these spacings appear in sections where the plane of the membranes has been tilted well away from the axis of the electron beam. At such angles, the projected images of several membranes overlap, and regular lattice lines could appear only if the internal details of those membranes could be superimposed. Such observations provide indirect proof for the contention that all membranes of a *Rh. viridis* thylakoid stack share a common orientation.

Direct proof is provided by the freeze-fracture image in Fig. 5. In this micrograph, the orientation of internal lattice in four membranes of a thylakoid stack are visible, and they

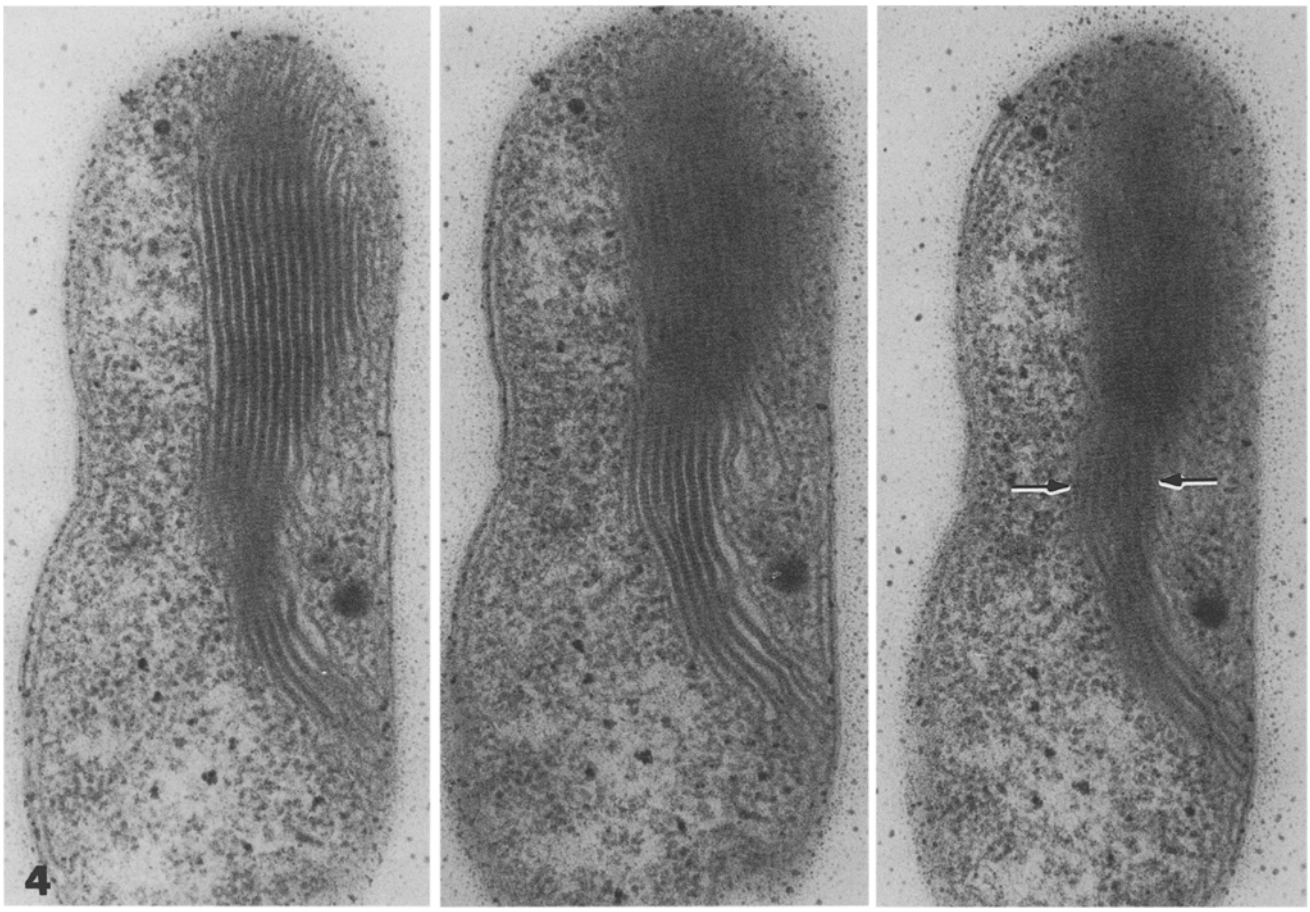

Fig. 4. An individual stack of *Rh. viridis* photosynthetic membranes sectioned obliquely and then tilted through various angles. The axis of tilt is vertical. The three micrographs, starting with the left hand image, have been through angles of 25°, −4°, and −24°, respectively. As a stack of membranes is tilted away from the perpendicular, regular striations appear at a right angle to the plane of the membrane (see *arrows*). The production of such striations is caused by two factors: 1) the regular lattice substructure of the membrane; and 2) the superposition of lattice from several membranes as the stack is tilted toward the oblique. This superposition could only produce regular striations if all the lattices of a stack were in alignment with each other. Magnification: 73,000

all match precisely. Naturally, the presence of such a match in one micrograph in which four thylakoids are visible does not prove common orientation. However, we have been unable to find a single example of a cell in which stacks of different orientation could be observed. When these observations are combined with the thin section images seen in Figs. 2–4), strong evidence is provided that the different membranes of a stack do in fact share a common orientation of membrane subunits.

The interthylakoid space

As seen in the earlier figures, one of the obvious attributes of the *Rh. viridis* photosynthetic membrane is its tendency to form stacks of closely apposed membranes. We have examined the spacing between adjacent membranes using three preparation techniques for electron microscopy: Thin sectioning, negative staining, and freeze etching. Representative views of membranes prepared with each of these techniques are seen in Fig. 6. Measurements have been made on these and similar micrographs to determine the spacings observed between pairs of membranes. These measurements are summarized in Table 1. Although these measurements vary considerably with different preparative techniques, the maximum value noted for a pair of photosynthetic membranes is 201 Å. This value is of particular interest because of recent data regarding the size and shape of the *Rh. viridis* photosynthetic reaction center. We will consider that data in the next section.

Table 1. Measurements of spacings in the *Rh. viridis* photosynthetic membrane

Parameter	T. S.	F. F.	N. S.
A	5.8 ± 0.8	9.1 ± 0.6	8.7 ± 1.7
B	16.0 ± 0.9	20.1 ± 1.0	–
C	4.2 ± 0.7	1.8 ± 0.1	–

Parameters refer to the dimensions noted in Fig. 8. A is the apparent thickness of a single membrane, B the thickness of a membrane pair, and C the spacing between two adhering membranes in a stack. All measurements are given in nanometers ± standard deviations, and are the mean values for 10 measurements

Discussion

Orientation of membrane lattices

The regular substructure of the *Rh. viridis* photosynthetic membrane has been well-described by a number of studies

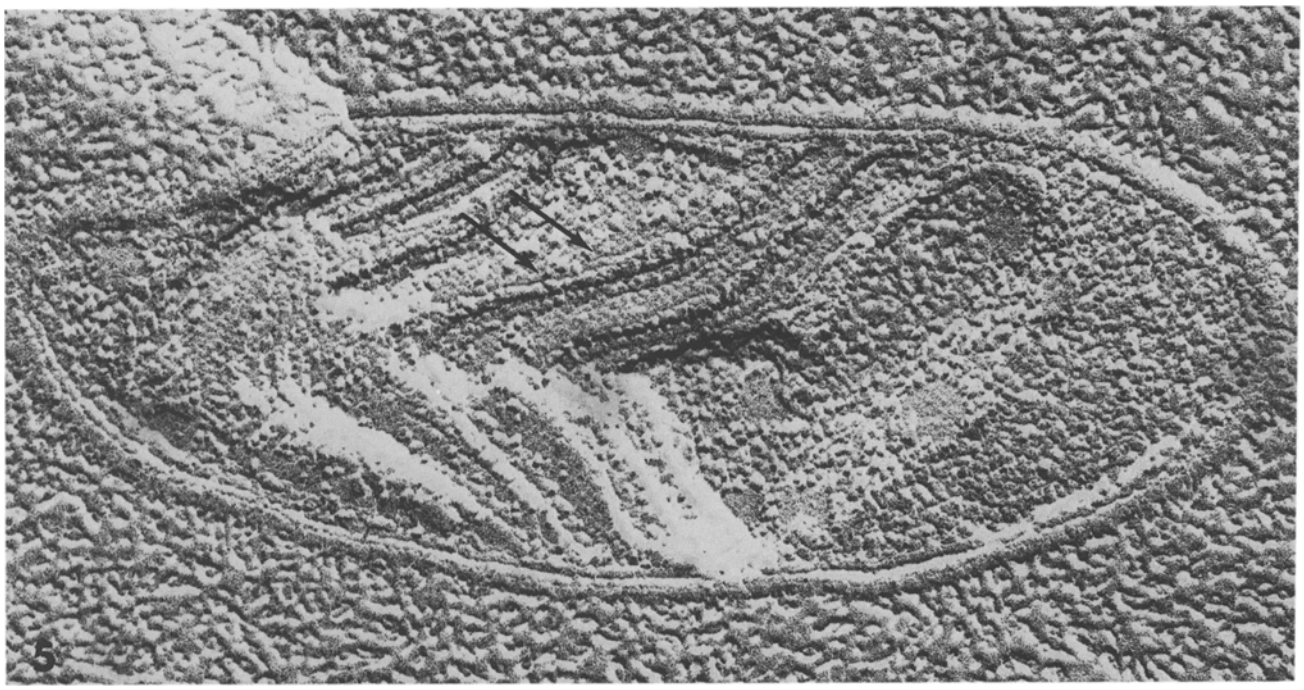

Fig. 5. This freeze-fracture image of an intact cell shows the direct alignment of lattice orientations on at least six individual membranes. The alignment of lattice orientation on each membrane (*arrows*) is consistent with structures observed in oblique sections of the membrane shown in Figs. 2–4. Magnification: 104,000

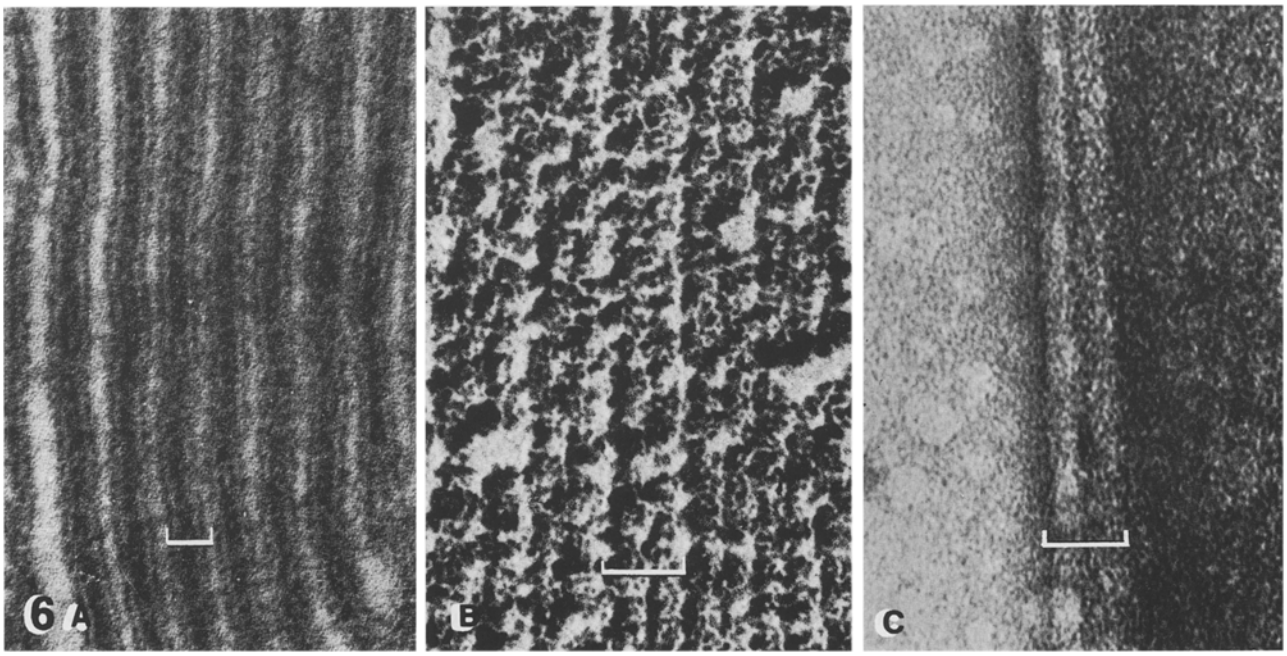

Fig. 6. Detailed views of the *Rh. viridis* photosynthetic membrane prepared by: **a** thin-sectioning, **b** freeze-fracture, and **c** negative staining. The pairing of membranes to form small stacks is most evident in **a** and **b**, where stacks are enclosed in small brackets near the bottom of each micrograph. Measurements of the dimensions of membranes and stacks are summarized in Table 1. Magnification: 375,000

(Engelhardt et al. 1983; Miller 1979; Wehrli and Kubler 1980; Stark et al. 1984; Miller 1982; Giesbrecht and Drews 1966). The basic structure of each individual subunit in the membrane includes a large central structure which is exposed at both surfaces of the membrane, and a ring of smaller structures which surround it (Engelhardt et al. 1983; Miller 1979; Wehrli and Kubler 1980; Stark et al. 1984). Given the size of the central structure and the regularity of the

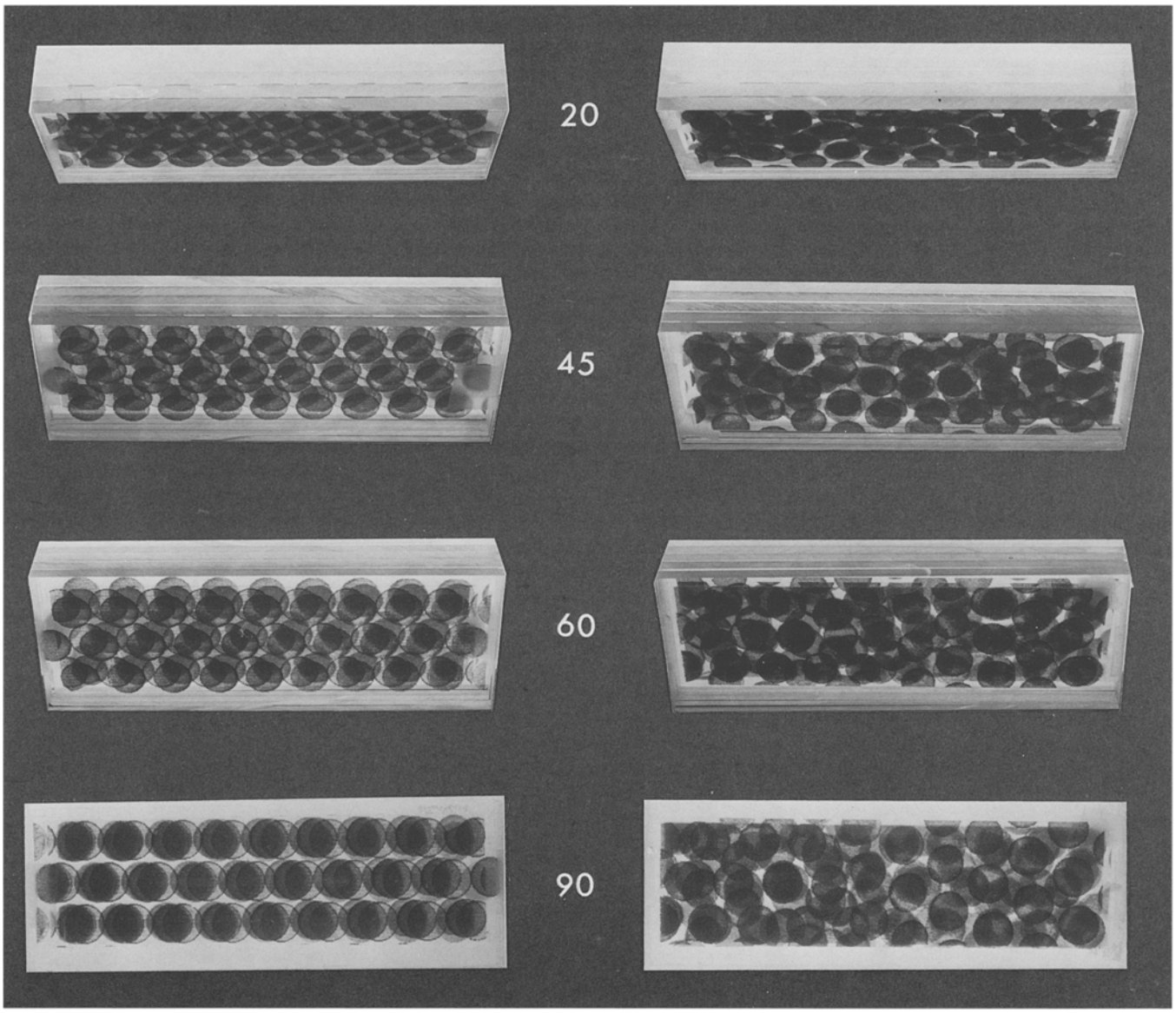

Fig. 7. Models illustrating the effects of tilting on observation of thin-sectioned membranes. A model of four membranes was produced by sandwiching regular patterns of black circles between lucite sheets. One model contained sheets with a common orientation. Another model contained four sheets with regular hexagonal lattices oriented in different directions with respect to each other. Photographs of the model are shown at 90°, 60°, 45°, and 20° from the direction of observation. Although superposition of the structure of individual membranes occurs in both samples, only sample with a common orientation produces a regular pattern. A similar effect can be seen in Figs. 2, 3 and 4

membrane, it is not surprising that the regular lattice of the membrane can be observed in electron micrographs made from thin sections of the cell.

However, there is nothing about the basic substructure of the membrane which requires that the lattice arrangements of adjacent membranes be in perfect register with each other. Nonetheless, that is precisely what Figs. 1–5 show. Tilting of cross-sectioned membranes in the electron microscope should bring about a superposition of the images of several membranes. We modeled this effect with a series of transparencies sandwiched between sheets of lucite plastic to simulate the substructure of individual membranes in a stack. One model was constructed from sheets which shared a common orientation (although they were not in precise register with each other) and the other model from sheets which were randomly oriented.

As shown in Fig. 7, the superposition of lattices in oblique membrane stacks produces regular striations only when all the sheets share a common orientation. Because such striations are observed in thin sections of the *Rh. viridis* cell, we can conclude that the individual membranes of a stack do indeed share a common orientation.

Direct confirmation of this conclusion is provided by Fig. 5, where the regular lattices of at least six membranes can be observed. All these membranes share the same common orientation. At present, we cannot offer an explanation for this common orientation. One might argue that attractive forces between adjacent membranes force the membrane to assume a common orientation, in order to allow the protruding central structure from each membrane to interlock. While this explanation makes mechanical sense, we do not know the nature of the attractive force between

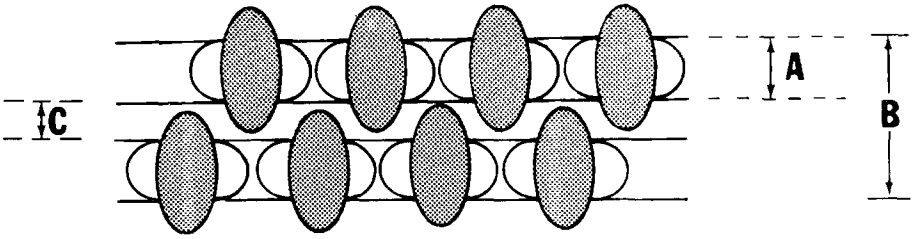

Fig. 8. Highly diagrammatic model illustrating some aspects of the organization of the *Rh. viridis* photosynthetic membrane. The dimensions of individual reaction centers (Michel et al. 1984) and the overall shape of the membrane subunit (Miller 1982) are taken from published data. When two membranes with such substructure are apposed at the distances determined in this report, the reaction centers must interdigitate with those on the adjacent membrane, as shown. Reaction centers are drawn as shaded ellipses. Each reaction center is shown surrounded by a ring of light-harvesting polypeptide. The significance of this close contact is not understood. However, the close apposition of regular lattices does not appear to allow for the inclusion of a coupling factor (ATPase) into the regular membrane lattice, presenting a problem in understanding the details of membrane function

the membranes, and find it difficult to build models around the phenomenon without such understanding.

A comparison with the reaction center structure

The atomic-level model for the organization of the *Rh. viridis* reaction center (Michel 1983; Michel et al. 1984) makes it possible to impose the dimensions of the reaction center directly on the observations which we have made on the membrane *in situ*. The dimensions reported by Michel et al. (1984) for the *Rh. viridis* reaction center suggest that the complex is 120 Å in length, with a cross-sectional profile measuring 55×35 Å. In Fig. 8, we have taken these dimensions and drawn them against the model reported by Miller (1982) for the membrane and our measurements from this paper for the spacing of membrane pairs *in situ*. When this exercise is carried out, each reaction center spans its own membrane and extends far enough from the membrane surface to nearly touch the surface of the apposing membrane. Indeed, this model suggests that there is a substantial densitiy of reaction center in the space between two stacked membranes, perhaps accounting for the electron-dense character of this region after it has been stained in the thin sectioning technique.

When one compares the reaction center model with the membrane structures observed by various techniques in this way, there seems to be very little room for any other structures at the surface of the membrane in stacked regions. This observation presents a problem in terms of understanding the energy-transduction mechanism of the *Rh. viridis* photosynthetic membrane. Higher plants (Miller and Staehelin 1976) and photosynthetic bacteria (Reed and Raveed 1972) both contain large protein complexes at the surface of their photosynthetic membranes which are involved in the synthesis of ATP. These membrane-bound ATPases, or coupling factors, are as much as 110 Å in diameter. They do not seem to fit in the space available between the stacks of *Rh. viridis* membranes, and therefore may be absent from these regions of the membrane.

In the higher plant thylakoid, coupling factor molecules are also absent (Miller and Staehelin 1976) from stacks of membranes, but are found on membrane surfaces in non-stacked regions. It is possible that such an arrangement exists in *Rh. viridis*? At this point it is not possible to say, but some of our observations seem to argue against it. Stereo images of sectioned cells (Fig. 2) and freeze-fractured images of the cell might reveal regions where the regular membrane lattice was not present, if such regions existed. To date we have not been able to observe non-lattice regions in any of the thylakoids under study.

It is also worth noting in this regard that studies in our lab and others (Jacob and Miller 1983; Jay et al. 1984) have failed to reveal any polypeptides associated with purified photosynthetic membranes from *Rh. viridis* which might be associated with a membrane-bound coupling factor.

Acknowledgements. This work was supported by a grant from the National Institutes of Health (GM 28799).

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Received January 17, 1985/Accepted May 17, 1985