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Variable Planar Particle Arrangements in the Photosynthetic Membrane of *Rhodopseudomonas viridis*

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Abstract. The thylakoids of *Rhodopseudomonas viridis* have been studied by freeze-fracturing whole cells. Depending on growth conditions and treatment before freezing, three different types of particle arrangements in the photosynthetic membrane are reported: a random arrangement, an isometric (quadratic) lattice arrangement with a lattice constant of 12.5 ± 0.8 nm, and a hexagonal lattice arrangement with a lattice constant of 12.5 ± 0.8 nm.

Key words: Photosynthetic bacteria – Electron microscopy – Planar lattices

The hexagonal particle arrangement of *Rhodopseudomonas viridis* thylakoids reported 14 years ago (Giesbrecht and Drews 1966), Garcia et al. (1968) have recently been reinvestigated by Miller (1979) and Wehrli (1979, 1980). We observed total cells with TEM after growth under different conditions and freeze-fracturing.

In spite of the frequent occurrence of hexagonally ordered particle arrangements seen in negative staining micrographs of isolated thylakoids, whole bacteria frozen under daylight rarely show extensive and undisturbed membrane crystals in freeze-fracture replicas (Miller 1979). This may be due to unusual fracture properties of the thylakoid stacks.

We have increased the yield of views on thylakoids with ordered particle arrangements by a special treatment of the bacteria prior to freezing, involving darkness after the harvest and a temperature change from 4° C to near the growth temperature (see below). This is supposed to loosen the contact between the thylakoids of the stack. Since the bacteria were grown in bottles with a diameter of 10 cm, the light intensity becomes extremely inhomogenous with increasing density of the culture. We measured the light intensity (lux) at the surface of the bottle, and we chose 3,000–5,000 lux as 'high-light conditions' and 100–400 lux as 'low-light conditions'. The cultures were grown at 30–33° C, referred to as 'high-temperature conditions', and at 24–26 °C, referred to as

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'low-temperature conditions'. The bacteria were washed three times in 50 mM Tris-HCl buffer, pH 8, and sedimented (5,000 g, 15 min, 10° C) in a fixed angle rotor. All subsequent steps were performed under dim green light or in the dark.

The bacteria were resuspended in Tris HCl buffer (50 mM, pH 8) and kept at 4° C for 4 h, then sedimented again; the supernatant was discarded and the pellet was kept at 24° C or 33° C for 20 min. Drops of the pellet were transferred to gold specimen supports and kept at 24° C or 33° C for 5, 10, and 15 min. The bacteria were then frozen in liquid propane and fractured in a Balzers freeze-fracturing device BA 360 at an object temperature of −100° C and a knife temperature of −150° C.

Freezing the whole bacteria was done without cryoprotectants, since there were no indications for freezing artefacts.

Micrographs of bacteria kept at daylight and at room temperature prior to freezing showed almost no thylakoid surfaces or fracture faces. Cross-fractured thylakoid stacks which were densely packed were predominant.

Freezing of the bacteria in the dark after keeping the cells near to growth temperature, as described above, caused swelling of the thylakoid lumen. Under favourable swelling conditions extensive views of what we suppose to be surfaces of thylakoids became visible in these samples. Three types of particle arrangements can be classified:

Randomly distributed particles as shown in Fig. 1.

An isometric lattice arrangement with a lattice constant of 12.5 ± 0.8 nm as shown in Fig. 2 (reported by Stange et al. 1980).

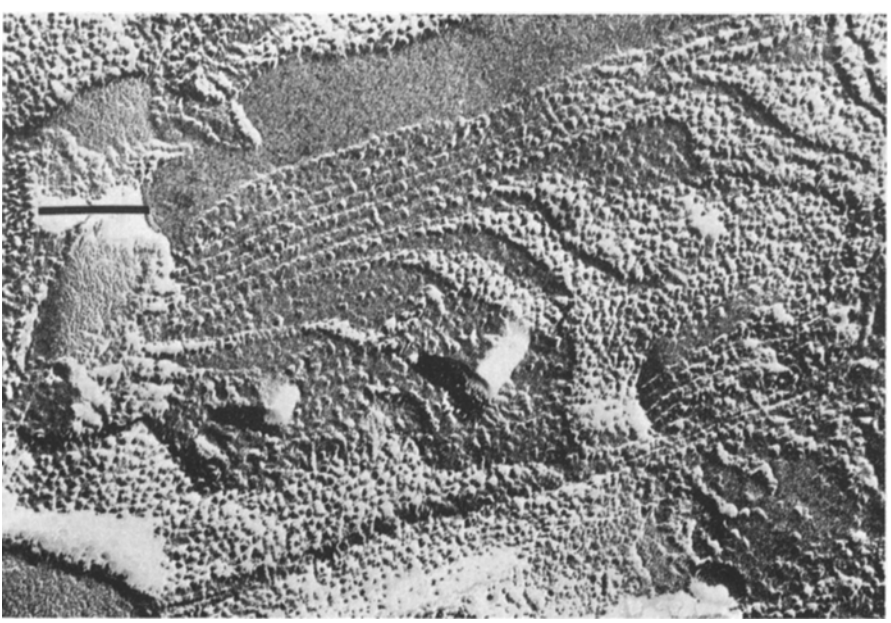

Fig. 1. Bar represents 0.2 μ

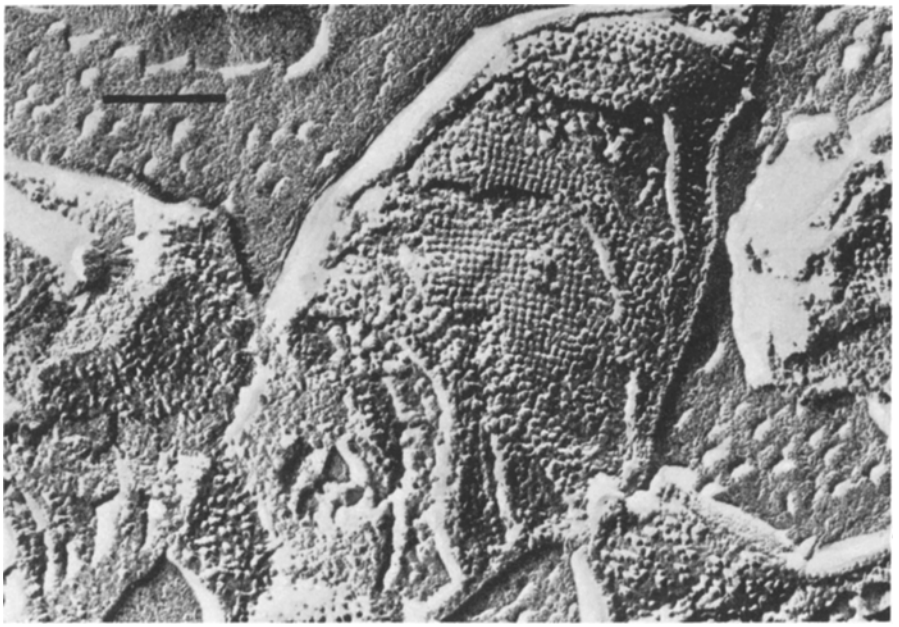

Fig. 2. Bar represents 0.2 μ

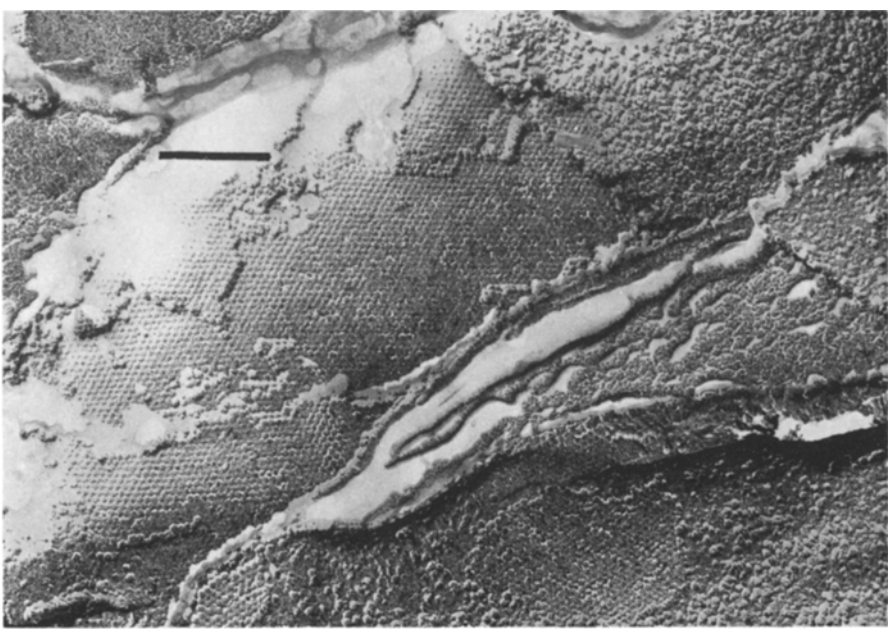

Fig. 3. Bar represents 0.2 μ

A hexagonal lattice arrangement with a lattice constant of 12.5 ± 0.8 nm as shown in Fig. 3.

Stacked thylakoids with hexagonal lattice arrangements often show parallel lattice axes of neighboured thylakoids as seen in Fig. 3. This indicates a tight interthylakoid particle contact and a three-dimensional lattice in the stack.

The occurrence of the different types of particle arrangements was found to depend on growth conditions, but may also be influenced by the degree of swelling of the thylakoid lumen.

We have observed all the different particle arrangements within one bacterium, but there were other examples showing exclusively randomly arranged particles or randomly and hexagonally arranged, or randomly and isometric arranged particles.

We suppose that the hexagonal lattice arrangements are favoured by growth at 'low light' and 'high temperature conditions'.

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