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Bacteriochlorophyll Synthesis and the Ultrastructure of Wild Type and Mutant Strains of *Rhodopseudomonas spheroides*[1]

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ABSTRACT

The ultrastructure of sectioned cells of mutant and wild type *Rhodopseudomonas spheroides* has been examined by electron microscopy. The characteristic vesicles associated with the presence of bacteriochlorophyll were found in wild type cells grown with low aeration. These were also found in mutant TA-R which forms bacteriochlorophyll under high aeration. None of the mutants with blocks in bacteriochlorophyll synthesis contained intracytoplasmic membrane. These included mutant 8-17 which accumulates bacteriochlorophyllide but fails at the phytolation step. We conclude that the intact bacteriochlorophyll molecule, or some particular membrane protein associated with it, is needed for the development of the characteristic intracytoplasmic membrane system in *R. spheroides*.

The characteristic intracytoplasmic membranes seen by electron microscopy of photosynthetic bacteria are presumed to contain chlorophyll and other components of the photosynthetic apparatus (2, 9). Examination of cells grown under various conditions has shown that the concentration of bacteriochlorophyll is correlated with the amount of these membrane structures. In the case of Athiorhodaceae which can grow aerobically in the dark, the bacteriochlorophyll concentration can be varied by the degree of aeration or by altering the light intensity, and both types of approach have shown the parallel between pigment and membrane content. This raises the question of the role of the bacteriochlorophyll molecule in the formation and structure of the membranes and whether synthesis of the entire molecule is needed for their development.

In an attempt to answer some of these questions we have examined the ultrastructure of mutant strains of *Rhodopseudomonas spheroides* with blocks at various stages in bacteriochlorophyll synthesis. Some of these form magnesium-containing precursors when grown under low aeration and presumably fail at steps on the chlorophyll branch of the biosynthetic path (13). Mutant TA-R has also been examined; unlike the wild type this strain makes abundant bacteriochlorophyll when grown with high aeration (10).

MATERIALS AND METHODS

Organisms. The wild type strain of *R. spheroides* and the various mutants derived from it have been described previously (8, 10, 13). Their characteristics are summarized in Table I.

Stock cultures were maintained on yeast extract-malate-glutamate agar (8), either as stab cultures grown in the light (wild type and strain TA-R) or as slants incubated in the dark for those strains which did not make bacteriochlorophyll.

Growth. Cells were grown in malate-glutamate medium supplemented with 0.2% (w/v) Difco yeast extract. For growth with low aeration, Erlenmeyer flasks were filled to 80% capacity with growth medium, and shaken on a rotary shaker at 200 rpm in the dark; these conditions promote membrane and chlorophyll synthesis in the wild type (Table I). For growth with high aeration, cells were incubated in 500 ml volumes in 2-liter Fernbach flasks on a reciprocal shaker operating at 55 oscillations/min. Such conditions repress pigment synthesis in

Table I. *Summary of Characteristics of Wild Type and Mutants*

Strain	Growth[1] Condition	Chlorophyll Precursors Accumulated	Bacteriochlorophyll	Carotenoids	Chromatophore Formation
			nmoles/mg dry wt. of cells		
Wild type	Low aeration	Traces	4.8	4.0	+
	High aeration	Nil	<0.1	<0.1	–
L-57	Low aeration	Nil	ND[2]	ND	–
8-29	Low aeration	2-Devinyl-2-α-hydroxyethyl chlorophyllide *a* (P-662)	ND	10.0	–
8-17	Low aeration	Bacteriochlorophyllide (P-770)	ND	1.0	–
TA-R	High aeration	Nil	1.5	1.8	–

[1] All cultures were grown in the dark.
[2] Not detectable.

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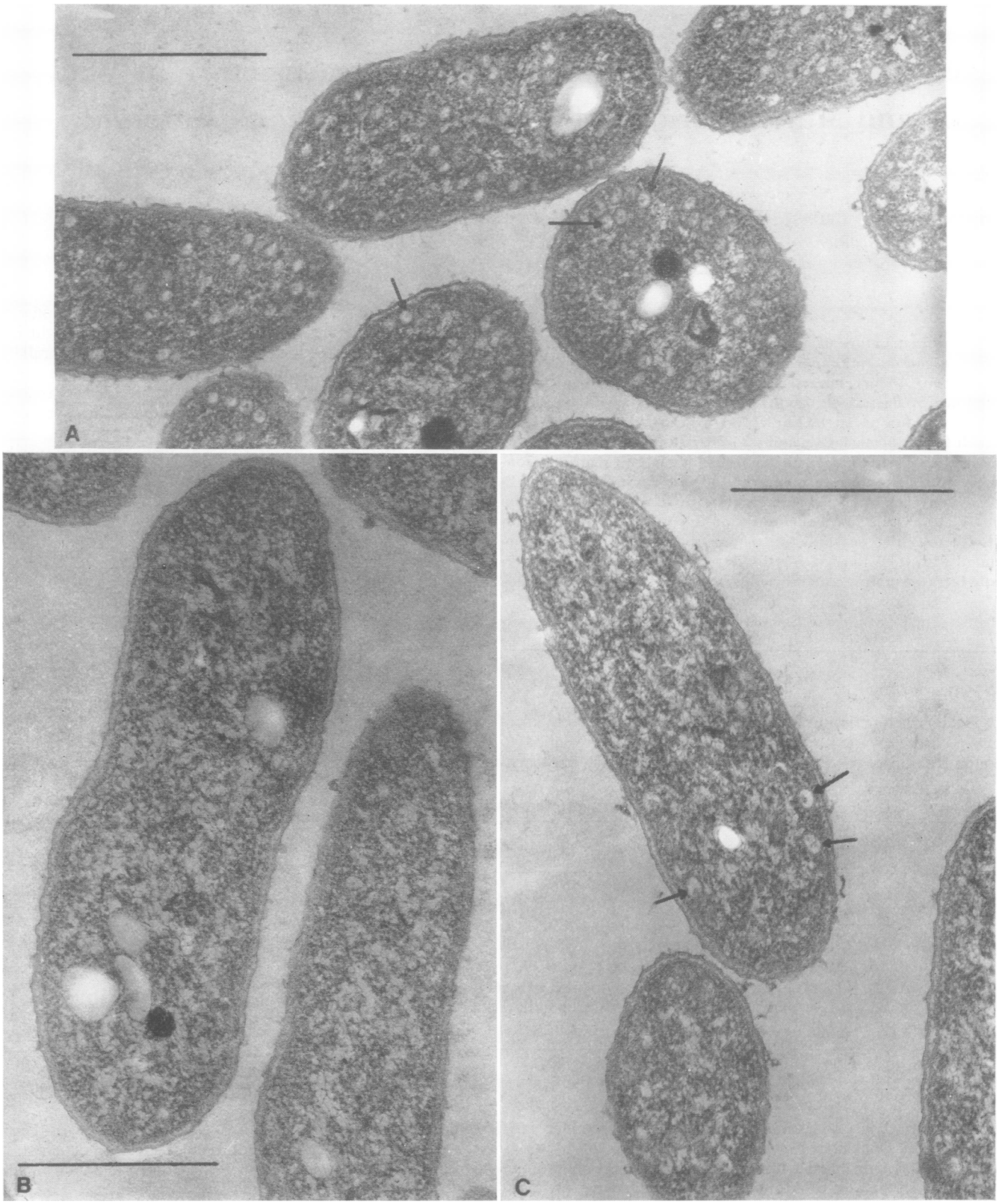

FIG. 1. A: Electron micrograph of wild type *R. spheroides* grown with low aeration. Arrows denote chromatophores. Bar represents 0.5 μ. B: Micrograph of wild type *R. spheroides* grown with high aeration. Note the absence of chromatophores. Bar represents 0.5 μ.C: Micrograph of mutant TA-R of *R. spheroides* grown with high aeration. Arrows denote chromatophores (compare to Fig. 1B). Bar represents 0.5 μ.

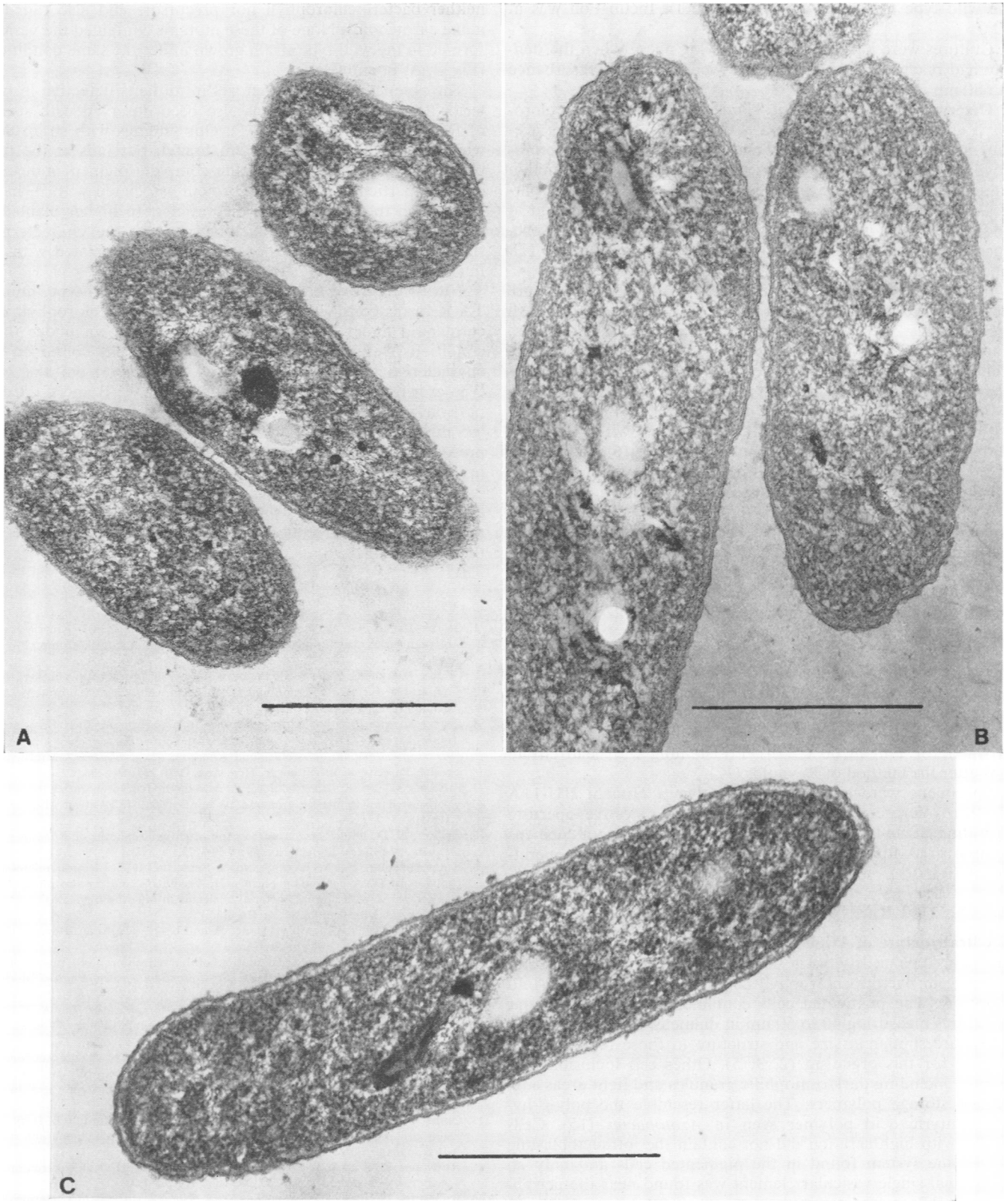

FIG. 2. Electron micrographs of mutant strains of *R. spheroides* grown with low aeration. A: L-57; B: 8-29; C: 8-17. Note the absence of chromatophore membranes in these strains unable to synthesize bacteriochlorophyll. Bar represents 0.5 μ.

the wild type to very low levels (Table I). Incubation was at 30 C.

Cultures were harvested in the mid-log phase when the density had reached 0.5 to 0.6 mg dry weight per ml (absorbance at 680 nm = 1 to 1.2).

Determination of Pigment Concentration. Bacteriochlorophyll and carotenoids were extracted from the cells with acetone-methanol (7:2) and the concentration of bacteriochlorophyll was determined at 770 nm using the millimolar extinction value of 76 (1, 2). The mixture of carotenoids formed, particularly in the presence of oxygen, had a broad maximum at 484 nm. Their concentration was calculated from the absorbance at 484 nm using the millimolar extinction value of 128 calculated from the data of Cohen-Bazire *et al.* (1).

Preparation of Cells for Electron Microscopy. Culture samples were centrifuged and the cells resuspended in fresh medium plus 2.5% (v/v) glutaraldehyde (Fisher Scientific Co., Fair Lawn, N. J.), and fixed for 30 min at room temperature. Cells were washed several times with 0.1 M potassium phosphate buffer (pH 7.2), and fixed in 1% (w/v) osmium tetroxide (Mallinckrodt Chemical Works, Los Angeles, Calif.), for 12 to 14 hr at room temperature. Cells were washed with phosphate buffer, followed by Michaelis buffer, pH 7.2 (6) and centrifuged into a small pellet (4 mm) in a Beckman Microfuge (Beckman Instruments, Palo Alto, Calif.). An equal volume of melted 2% (w/v) Difco agar in Michaelis buffer was added and mixed with the cell pellet. The resulting solidified pellet was cut into 0.5-mm blocks, and transferred to 0.5% (w/v) uranyl acetate in Michaelis buffer for 1 hr. Agar blocks were dehydrated in a graded series of acetone-water, infiltrated with Vestopal (M. Jaeger, Cie., Vezenaz, Geneva, Switzerland), and cured in gelatin capsules at 60 C for 24 hr.

Thin sections were cut on an LKB Ultratome (LKB Instruments, Stockholm, Sweden) with glass knives. Silver to gray sections were mounted on 200 mesh copper grids (Ted Pella Inc., Tustin, Calif.) covered with net films (4) and carbon-stabilized. Sections were stained with saturated uranyl acetate in water for 1 hr at 60 C (14). Poststaining with lead citrate was after the method of Reynolds (12).

Specimens were examined with either a Hitachi HU-11A electron microscope fitted with a 50-μm objective aperture operating at an accelerating voltage of 75 kv, or a Siemens Elmiskop 1A fitted with a 50-μm objective aperature, operating at 80 kv.

RESULTS AND DISCUSSION

Ultrastructure of Wild Type. An examination was made of the wild type cells grown with low and high aeration and differing in bacteriochlorophyll content by more than 40-fold (Table I). The pigmented cells contained numerous vesicular structures measuring 40 to 60 nm in diameter (Fig. 1A, arrow). These are similar in size and structure to those reported previously for this organism (2, 3, 5). Other cell inclusions are evident including dark osmophilic granules and light areas suggesting storage polymers. The latter resemble the poly-β-hydroxybutyric acid polymer seen in *Azotobacter* (15). Cells grown with high aeration did not contain the intracytoplasmic membrane system found in the pigmented cells and only an occasional single vesicular element was found near the periphery (Fig. 1B).

Ultrastructure of Bacteriochlorophyll-less Mutants. Mutants unable to form bacteriochlorophyll were grown under low aeration for ultrastructural examination. Mutants 8-29 and 8-17 with blocks on the magnesium branch of the tetrapyrrole biosynthetic path accumulated precursors under these conditions; both formed carotenoids (Table I). Mutant L-57 formed neither bacteriochlorophyll nor precursors and also failed to make carotenoids. None of these mutants contained the vesicles formed in the wild type strain grown under the same conditions (Fig. 2, A, B, and C).

The absence of these structures in mutants defective in bacteriochlorophyll synthesis has also been observed with strains of *Rhodospirillum rubrum* (11). Our findings with strain 8-17 which has the entire magnesium branch but fails at the final phytolation step, suggest that the complete pigment molecule, or some particular membrane protein associated with it, has a key role in the formation of the vesicles. In higher plants the organization of thylakoid membranes into the characteristic structure of the mature chloroplast is also associated with the final phytolation step (7, 16).

Ultrastructure of Strain TA-R. Unlike the wild type, mutant TA-R forms considerable amounts of bacteriochlorophyll and carotenoid under high aeration, and cells grown under these conditions were therefore examined. Such cells contained the characteristic vesicles and differed strikingly from the wild type grown under the same condtions (Fig. 1, B and C). Comparison with Figure 1A shows that the vesicles in TA-R were less numerous than in wild type cells grown with low aeration; presumably this can be attributed to the 3- to 4-fold difference in bacteriochlorophyll content (Table I).

The presence of vesicles in this mutant when grown with abundant aeration shows that formation of bacteriochlorophyll rather than oxygen concentration is critically important for their formation.

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