Three-dimensional Ultrastructure of a Unicellular Cyanobacterium

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ABSTRACT The first complete three-dimensional ultrastructural reconstruction of a cyanobacterium was accomplished with high-voltage electron microscopy and computer-aided assembly of serial sections. The precise arrangement of subcellular features within the cell body was very consistent from one cell to another. Specialized inclusion bodies always occupied specific intracellular locations. The photosynthetic thylakoid membranes entirely surrounded the central portion of the cytoplasm, thereby compartmentalizing it from the rest of the cell. The thylakoid membranes formed an interconnecting network of concentric shells, merging only at the inner surface of the cytoplasmic membrane. The thylakoids were in contact with the cytoplasmic membrane at several locations, apparently to maintain the overall configuration of the thylakoid system. These results clarified several unresolved issues regarding structure-function relationships in cyanobacteria.

Cyanobacteria (blue-green algae) are phototrophic microorganisms that carry out a form of oxygenic photosynthesis similar to that of green plants. The internal organization of the cyanobacterial cell is prokaryotic, yet these organisms are considerably more structurally complicated than most other types of bacteria. The ultrastructure of cyanobacteria has been studied extensively, and much is known about their basic subcellular features, the structural differences among the various genera and groups, and the effects of environmental factors on internal structures. (For reviews, see references 1–3.) Little is known, however, about how the ultrastructural features of cyanobacteria are arranged three-dimensionally within the cell. Most speculations concerning the three-dimensional architecture of these organisms have come about through extrapolation of the information in randomly cut, individual thin sections. There have been no reports describing three-dimensional reconstructions of entire cyanobacterial cells by serial sectioning or any other approach. Reconstructions of this type are needed, however, because they (*a*) would eliminate the need for extrapolation, (*b*) can resolve a number of uncertain issues in regard to cyanobacterial ultrastructure (see below), and (*c*) may lead to a better understanding of structure-function relationships in the cyanobacterial cell.

Complete three-dimensional reconstructions of whole cells or cell organelles have been relatively rare because, until recently, the techniques for completing such reconstructions were cumbersome and time-consuming. However, the application of high-voltage electron microscopy (HVEM[1]) to biological materials has now made it possible to view serial thick (0.25–1.0 μm) sections without any significant loss in resolution (4–7). Since these sections are much thicker than conventional thin sections, fewer of them are required to cover a given sample volume during serial sectioning. Three-dimensional reconstructions have also been greatly facilitated by the development of computer programs designed to collect the information from serial thin or thick sections and then display it in three-dimensional form (8–16). This procedure has eliminated the need to prepare clay contour models, hand-drawn plastic sheets, etc. The cumulative effect of these recent technological advances has been to make three-dimensional reconstruction a feasible and efficient approach to ultrastructural research.

The purpose of the present study was to elucidate the three-dimensional arrangement of ultrastructural features in a cy-

[1] *Abbreviations used in this paper*: CARTOS, computer-aided reconstruction by tracing of sections; HVEM, high-voltage electron microscopy.

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anobacterium. *Agmenellum quadruplicatum*, a unicellular halotolerant cyanobacterium, was chosen for this study because of its relatively simple cellular morphology and because its basic ultrastructural features have been characterized thoroughly (17–22). In carrying out the general reconstruction of this organism, we concentrated on specific issues that either have not been addressed in the literature or have been the subject of confusion and controversy. These included: (*a*) the consistency of three-dimensional arrangements from one cell to another, (*b*) the locations of specialized inclusion bodies, (*c*) the detailed arrangement of photosynthetic thylakoid membranes, and (*d*) the possibility that the thylakoid system was in direct contact with the cytoplasmic membrane. We describe here the complete three-dimensional architecture of *A. quadruplicatum* and discuss the significance of this information in relation to the above issues.

MATERIALS AND METHODS

Organism and Culture Conditions: *Agmenellum quadruplicatum* strain PR-6 was isolated and described by Van Baalen (23). It was grown in medium A, as described by Stevens et al. (24). Broth cultures were incubated at 39°C in a glass recirculating water bath. Continuous agitation and a carbon source were provided by bubbling of sterile 3% (vol/vol) CO_2 in air through the cultures. Illumination consisted of four (two on each side of the bath) F72T12 CW/HO fluorescent lamps providing an intensity of 380 μmol photons m^2 s^{-1} incident on each growth tube. Exponentially growing cultures were used for all investigations described below.

Preparation of Samples for Electron Microscopy: Two fixation procedures were used to prepare cells for thin or thick sectioning: a glutaraldehyde-osmium tetroxide technique (22) and a ruthenium red technique (21). Micrographs from the glutaraldehyde-osmium tetroxide procedure were chosen for illustrations in this report; the ruthenium red method gave equivalent results. Samples from both fixations were dehydrated through a graded ethanol series. Those to be used for conventional thin sectioning were embedded in Spurr's low-viscosity epoxy resin (25), whereas those to be used for thick sectioning were embedded in Poly/Bed 812 (Polysciences, Inc., Warrington, PA). Both resins were cured for 15 h at 65–70°C.

Serial thin sections were cut with a diamond knife, and serial thick (0.25-μm) sections were cut with glass knives. An LKB Ultratome III ultramicrotome was used in both cases. The serial sections were retrieved on carbon-formvar-coated multi-slot specimen grids, according to the technique of Anderson and Brenner (26). The thin sections were poststained for 15 min in 0.5% uranyl acetate (in 50% methanol) and for 2 min in 0.5% lead citrate (27). Poststaining times for the 0.25-μm thick sections were 60 min in the uranyl acetate and 30 min in the lead citrate.

Computer-aided Reconstruction of Serial Thin Sections: The serial thin sections were examined and photographed at an accelerating potential of 80 kV with a JEOL JEM-100S transmission electron microscope. Initially, serial sections through 20 groups or clusters of cells were photographed at low magnification (× 2,000). Enlargements of these micrographs were studied, and individual cells were selected for detailed reconstruction. Over 50 fully serial-sectioned cells were chosen, including representative examples of cells sectioned in different directions (i.e., some had been serially cross-sectioned, some had been longitudinally sectioned, etc.). These differing sectioning directions were selected as an internal control, since reconstructed cells would be expected to appear similar regardless of how they had been sectioned. Serial sections through each of the selected cells were rephotographed at magnifications sufficient to resolve the important cell features (× 10,000–20,000). These series of micrographs (50–75 sections for each cell) were then used in the reconstruction process described below.

Reconstruction of serially sectioned cells was done with the CARTOS (computer-aided reconstruction by tracing of sections) Loaner System developed in the laboratory of Dr. Cyrus Levinthal, Department of Biological Sciences, Columbia University. The CARTOS computer program has been described previously (11). The Loaner System was made available as a National Biotechnology Research Resource (see "CARTOS", Research Resources Information Center, United States Department of Health and Human Services, National Institutes of Health publication no. 81-2289). Prior to reconstruction, each of the series of electron micrographs produced above was converted to a 35-mm filmstrip in which each frame depicted one section in the series. The sections were optically aligned with respect to the *X* and *Y* axes (in three-dimensional space) as these filmstrips were produced. From projected images of each filmstrip, tracings of cell outlines and internal structures were entered into the computer system with the aid of an automatic digitalizing device. The computer was then instructed to display reconstructions of desired cell features or combinations of features on a video display system. These reconstructions were studied visually while they were rotated about the *X*, *Y*, and *Z* axes. Selected views were photographed directly from the display screen using Kodak LPD-4, Tri-X, and Ektachrome 160 films. Stereo pairs were generated by rotation and photographed to facilitate three-dimensional visualization of the structures.

HVEM of Thick Sections: The serial thick (0.25-μm) sections were examined and photographed at an accelerating potential of 1.0 MV with an AEI EM7 high-voltage transmission electron microscope. Serial sections cut in various directions through ~50 complete cells were photographed as stereo pairs to facilitate three-dimensional interpretation of the material in each section. Sections were also tilted about two perpendicular axes with the aid of a goniometer stage, to clarify or better understand the structure and arrangement of certain cell features. Appropriate views were then photographed as stereo pairs. Enlargements of all HVEM micrographs were studied extensively to analyze three-dimensional information contained therein.

RESULTS

Overall Arrangement of Ultrastructural Features

The principal ultrastructural features of *Agmenellum quadruplicatum* were visible in central, longitudinal sections (Fig. 1). All cells possessed an extracellular slime layer of glycocalyx (17) and a Gram-negative cell envelope identical to those found in other unicellular cyanobacteria (2). The interior of the cell included a central cytoplasmic region that appeared to be surrounded by a peripheral system of photosynthetic thylakoid membranes. Several specialized inclusion bodies were commonly present in addition to nuclear material and ribosomes. These included polyphosphate bodies (28–31), carboxysomes or "polyhedral bodies" (3, 32–34), glycogen granules or "α-granules" (3, 35, 36), lipid bodies or "β-granules" (3, 36, 37). As in previous investigations (21, 22), individual phycobilisomes (38, 39) were usually not resolved.

Both CARTOS reconstructions of serial thin sections and HVEM examination of serial thick sections were found to be quite helpful in determining the three-dimensional arrangement of the above features within the cell body. This arrangement proved to be quite consistent from one cell to another.

Nuclear material, polyphosphate bodies, and carboxysomes were located only in the central cytoplasmic portion of the cell. They were usually positioned along the central longitudinal axis and were never situated among the thylakoid membranes. Polyphosphate bodies and carboxysomes were interspersed with each other, so that direct contact between these two types of inclusions occurred frequently. Each cell contained an average of six carboxysomes and five polyphosphate bodies (ranges: 4–10 and 4–6, respectively). Polyphosphate bodies varied in diameter, but there was no correlation between their size and the number per cell. A CARTOS reconstruction illustrating only the carboxysomes within a cell is shown in Fig. 2. Reconstructions like this were especially helpful in enabling one to visualize easily the positions of specific intracellular features in three-dimensional space.

The central cytoplasmic region containing the inclusions described above was essentially an independent compartment within the cell, since it was completely surrounded by the innermost pairs of membranes in the thylakoid system. Direct contact between the cytoplasmic inclusions and these membranes was seldom seen, however, because the inclusions were almost always surrounded by ribosomes. For the most part, the ribosomes were also confined to the central cytoplasm and filled the areas not occupied by inclusions.

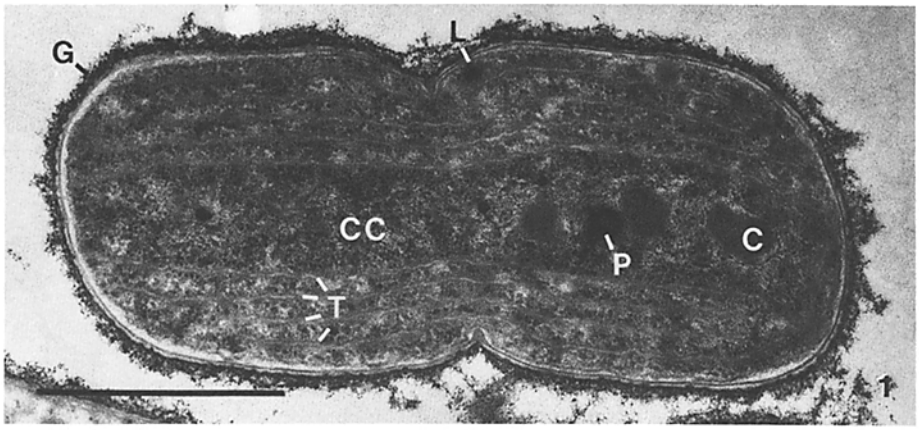


FIGURE 1 Electron micrograph of longitudinal thin section through *A. quadruplicatum* cell. C, carboxysome; CC, central cytoplasmic region; G, glycocalyx; L, lipid body; P, polyphosphate body; T, photosynthetic thylakoid membranes. Bar, 1.0 μm. × 31,800.

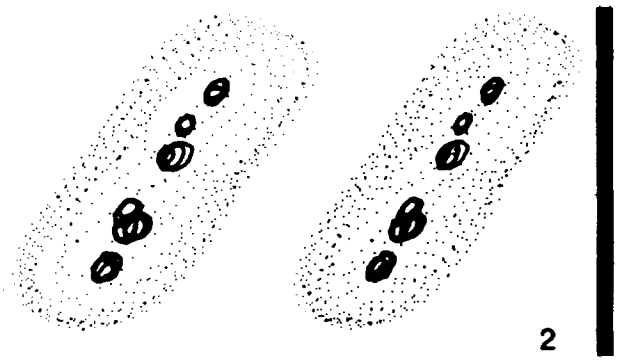


FIGURE 2 Stereo pair of CARTOS reconstruction, illustrating location of carboxysomes (solid circles) within a typical cell (outline represented by dots). Carboxysomes are positioned along central longitudinal axis of the cell.

FIGURE 3 Stereo pair of CARTOS reconstruction, illustrating location of lipid bodies (solid circles) within a typical cell (outline represented by dots). Compare peripheral position of these inclusions with central location of the carboxysomes shown in Fig. 2.

Glycogen granules, lipid bodies, and the thylakoids themselves were located only within the peripheral thylakoid membrane system that surrounded the central cytoplasmic region. Glycogen granules were dispersed between the membrane pairs throughout the entire thylakoid system, but lipid bodies were always positioned specifically between the cytoplasmic membrane and the outermost pair of thylakoid membranes. The average number of lipid bodies in each cell was 17 (range: 14–22). They were located about the entire periphery of the cell, including the hemispherical poles. A CARTOS reconstruction showing only the lipid bodies within a cell is shown in Fig. 3. Unlike the inclusions located in the central cytoplasm, lipid bodies were frequently seen to be in close association with the outermost pair of thylakoid membranes (Fig. 1).

General Three-dimensional Arrangement of the Thylakoid System

Reconstructions from serial thin sections with CARTOS were used to determine the general three-dimensional arrangement of the thylakoid membrane system in *A. quadruplicatum*. With the exception of one feature described below, this was also found to be quite consistent from one cell to another.

The complete thylakoid system included four to six pairs of membrane sheets that traversed the entire longitudinal axis of the cell. The sheetlike nature of the thylakoids and the extent to which these sheets spread throughout the cell were never apparent from individual thin or thick sections. These aspects of thylakoid structure were seen more readily in CARTOS reconstructions that were made by tracing a single thylakoid pair throughout the entire cell. Fig. 4 illustrates such a reconstruction made by tracing the innermost thylakoid pair facing the central cytoplasm. Both the sheetlike nature of the membranes and the fact that they entirely surround the central cytoplasmic region (as stated earlier) are evident.

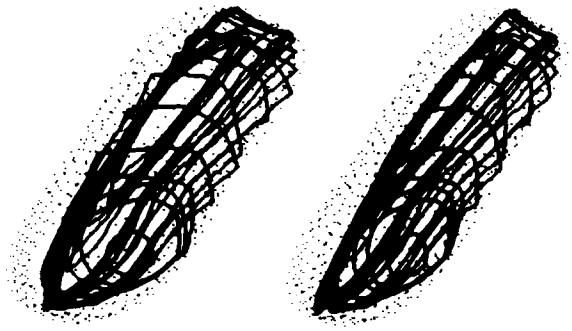


FIGURE 4 Stereo pair of CARTOS reconstruction, illustrating the innermost complete thylakoid (solid lines) within a typical cell (outline represented by dots). It is important to realize the contour lines delineating the thylakoid actually represent continuous, closed sheets of membranous material. Note that thylakoid entirely surrounds central cytoplasmic region of the cell and tapers toward each cell pole.

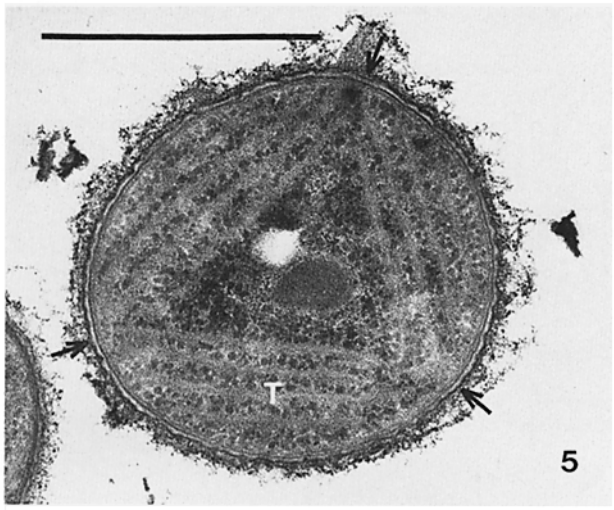


FIGURE 5 Electron micrograph of thin cross section through cylindrical portion of *A. quadruplicatum* cell. Thylakoids (*T*) tend to coalesce and approach edge of cell at three peripheral loci (arrows), producing a pattern similar to a series of concentric triangles. Bar, 1.0 μm. × 35,000.

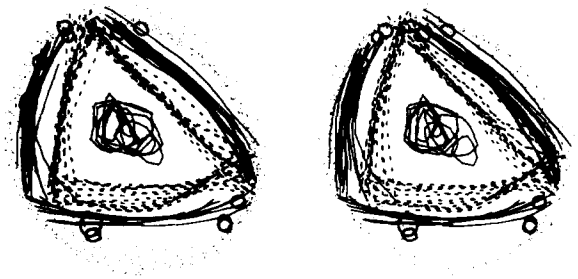


FIGURE 6 Stereo pair of CARTOS reconstruction, illustrating two complete thylakoid membranes within the cylindrical portion of a cell (outline represented by dots). Innermost thylakoid represented by dashed lines, outer thylakoid by solid lines. Other features include carboxysomes (central) and lipid bodies (peripheral).

The manner in which the 3–6 membrane sheets included in a complete thylakoid system related to each other in three-dimensional space was a primary concern of this study. Their arrangement in the uniformly cylindrical portion of the cell was determined first because it was relatively straightforward. It was most readily understood if one noted initially the pattern formed by the thylakoids in cross sections (Fig. 5). The thylakoid sheets tended to coalesce with each other and approach the edge of the cell at three peripheral loci. The resulting pattern was similar to a series of concentric triangles, except that they were not actually concentric at their corners. This pattern was consistent in all of the cross sections throughout the cylindrical part of the cell, thereby indicating that the thylakoid sheets extended straight through this region without changing their distance from the central longitudinal axis. A CARTOS reconstruction made from cross sections and depicting the placement of two complete thylakoid membranes in the cylindrical part of a cell is shown in Fig. 6. A model of the complete thylakoid system in the cylindrical part of the cell is shown in Fig. 7. This model was produced with the aid of both CARTOS reconstructions and HVEM-derived data on detailed thylakoid arrangements near the cytoplasmic membrane (see below).

The thylakoid arrangement described above was found to occur in ~70% of the cells with little or no variation. The rest of the cells possessed a slightly different arrangement that was also quite consistent from one cell to another. Cross sections through these two arrangements are compared diagrammatically in Fig. 8. In the second arrangement, the thylakoid sheets appeared to coalesce and approach the edge of the cell at four peripheral loci instead of three. Actually, they approached only the outermost thylakoid membrane at one of those four positions. This pattern, like the first one, was consistent in serial cross sections through the entire cylindrical portion of the cell. Therefore, a model of the second thylakoid arrangement would be similar to that in Fig. 7, except for its appearance in cross section. There was no correlation between thylakoid pattern and the cell division cycle of *A. quadruplicatum*. Cells with either pattern were found in all stages of cell division. No other patterns were observed in any of the cells, nor were any seen that could be interpreted as transition stages between the two types illustrated in Fig. 8.

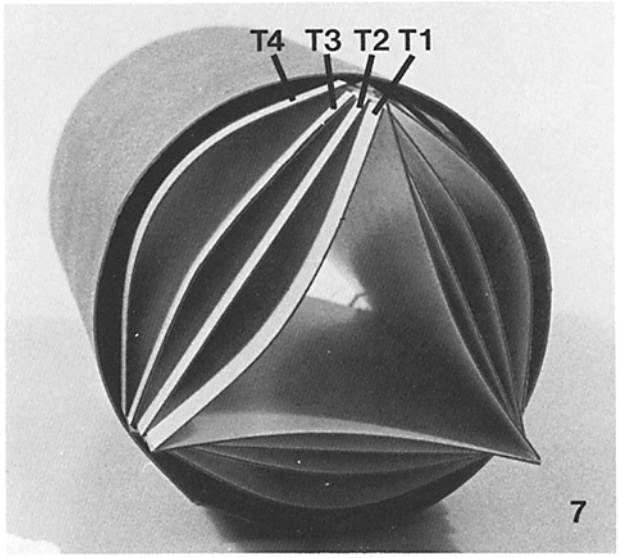


FIGURE 7 Three-dimensional, cut-away model of the thylakoid membrane system in the cylindrical portion of a typical cell. Four thylakoids (*T1*, *T2*, *T3*, and *T4*) have been included. (Based on CARTOS reconstructions and HVEM-derived data on detailed thylakoid arrangements near the cytoplasmic membrane; see text.)

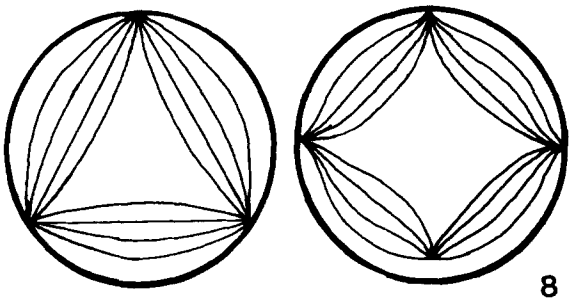


FIGURE 8 Diagrams comparing the two thylakoid patterns seen in cross sections through the cylindrical portion of *A. quadruplicatum* cells. At left is pattern seen in 70% of the cells (compare with three-dimensional model in Fig. 7); at right is variant pattern seen in remaining cells.

The arrangement of thylakoids in the hemispherical poles of the cell was more complicated than that in the cylindrical portion because it was affected by cell division. It was apparent from serial longitudinal sections and CARTOS reconstructions of pole areas (Fig. 9) that all of the thylakoid sheets tapered toward the tips of the cell. Examination of serial sections through pole areas further demonstrated that the innermost thylakoid pair always terminated at a single point at or very near the tip of the cell. It was the location of the outer thylakoid sheets that varied, and the variation was best understood from an examination of the pole areas in cells that had just completed division but had not yet separated from each other (Fig. 10). The thylakoid arrangement at the two "old" poles was always the same; all thylakoids tended to coalesce and terminate simultaneously at the tip of the cell. In the "new" poles, however, the outer thylakoids usually terminated near the cell periphery without quite reaching the tip. Individual cells (those no longer involved in cell division or separation) always possessed one pole with the first of the above thylakoid arrangements. The other pole exhibited either arrangement or any of a variety of transitions between the two.

FIGURE 9 Stereo pair of CARTOS reconstruction, illustrating appearance of the innermost thylakoid membrane (solid lines) in the pole of a typical cell (outline represented by dots). Thylakoid tapers to a single point at the cell tip (see also Fig. 4).

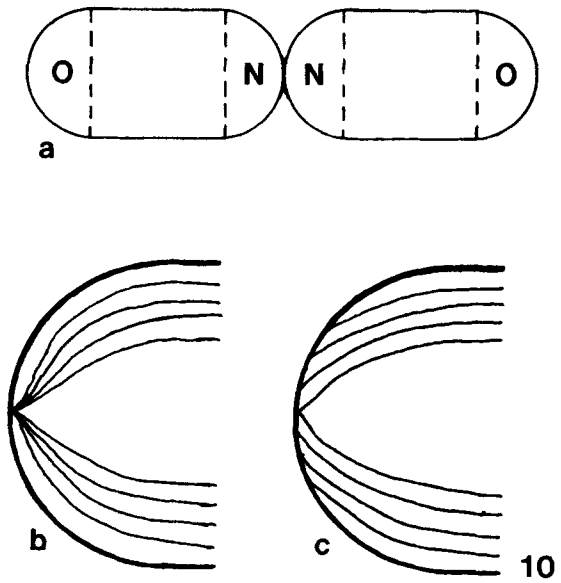


FIGURE 10 Diagrams illustrating the arrangement of thylakoid membranes in hemispherical poles of cells that have completed cell division but have not yet separated from each other. Diagrams represent theoretical longitudinal sections passing through the central longitudinal axes of the cells. (a) Outline of dividing cell pair. "Old" poles (*O*) are defined as those existing prior to cell elongation and division. "New" poles (*N*) are defined as those formed during division and which still face each other before cells separate; (*b*) Thylakoid arrangement in "old" poles; membranes merge at a common point near tip of cell; (c) thylakoid arrangement in "new" poles; membranes terminate at edge of cell without merging with each other.

To summarize visually the information obtained up to this point in the study, a few CARTOS reconstructions illustrating both the thylakoid membranes and the inclusion bodies were attempted. It was possible to increase the number of cell features that could be included without producing an excessively confused image by arbitrarily assigning colors to the individual thylakoids and inclusion bodies. Reconstructions made in this way were especially helpful in visualizing the arrangement of entire cells (Fig. 11).

Detailed Arrangement of the Thylakoid System with Respect to the Cytoplasmic Membrane

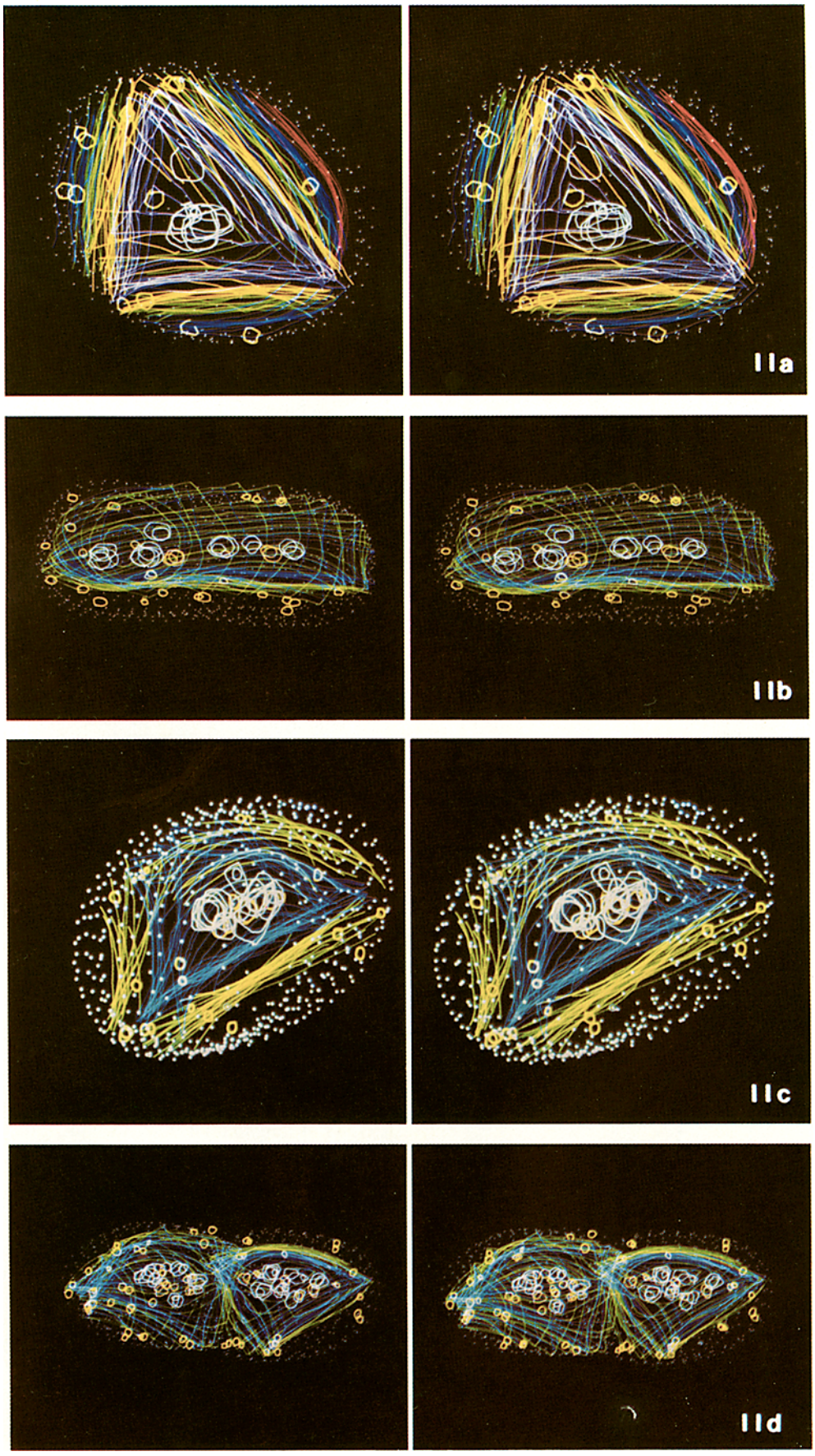
CARTOS reconstructions indicated that thylakoid sheets tended to converge and approach the periphery of the cell, but these reconstructions lacked the resolution needed to see the precise details in such structurally complex areas. HVEM of thick sections served to clarify the thylakoid arrangements in these areas and to ascertain whether the thylakoids contacted the cytoplasmic membrane.

The thylakoids in the cylindrical portion of the cell almost always intersected with each other at a common point on or near the surface of the cytoplasmic membrane (Fig. 12). In most cases, the thylakoids actually did contact the inner surface of the cytoplasmic membrane at this common intersection point (Fig. 13). Contact occurred at three peripheral loci in both of the thylakoid arrangements described earlier. (The inner thylakoids also intersected with the outermost thylakoid at a fourth locus in the second arrangement.) The arrangements were consistent in serial thick cross sections (or near cross sections) throughout the entire cylindrical portion of the cell. However, it was often necessary to tilt the thick sections about two perpendicular axes to see clearly the thylakoid arrangement and the contact with the cytoplasmic membrane. This tilting apparently compensated for slight differences in the direction of sectioning, since these features were seen clearly in some sections without tilting. Serial thin cross sections through the cylindrical portion of the cell were also examined with regard to the detailed thylakoid arrangement near the cytoplasmic membrane. The arrangement was the same as seen in thick sections, except that contact with the cytoplasmic membrane was seen much less frequently. The contacts, then, apparently were intermittent rather than continuous along the length of the cell.

The detailed thylakoid arrangement at the cell poles was studied in longitudinal thick sections. The innermost thylakoid sheet always terminated and contacted the cytoplasmic membrane at the tip of the cell. One or more of the outer thylakoids also terminated at this point in some cases. The thylakoid system of *A. quadruplicatum*, then, was in contact with the cytoplasmic membrane at both poles and at intermittent points along three lines that traversed the length of the cell. The data were insufficient to determine the spacing between the intermittent contact points or the consistency thereof. The three-dimensional structure of these contact points is illustrated along with the overall arrangement of a typical cell in Fig. 14.

DISCUSSION

This study elucidated for the first time the complete three-dimensional arrangement of ultrastructural features in a cy-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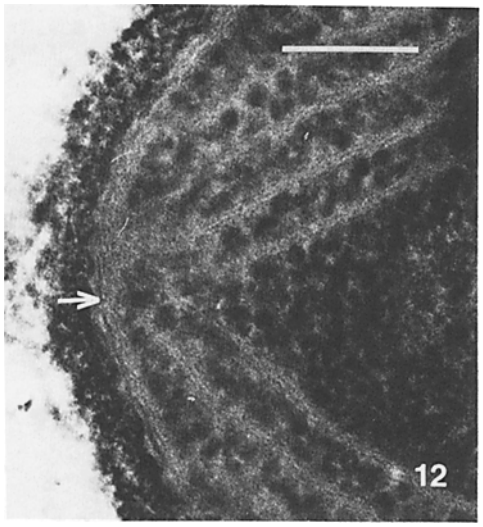

FIGURE 12 High-voltage electron micrograph of thick (0.25-μm) cross section through cylindrical portion of *A. quadruplicatum* cell, illustrating tendency of thylakoid membranes to converge at or near the inner surface of the cytoplasmic membrane (arrow). Bar, 0.25 μm. × 68,000.

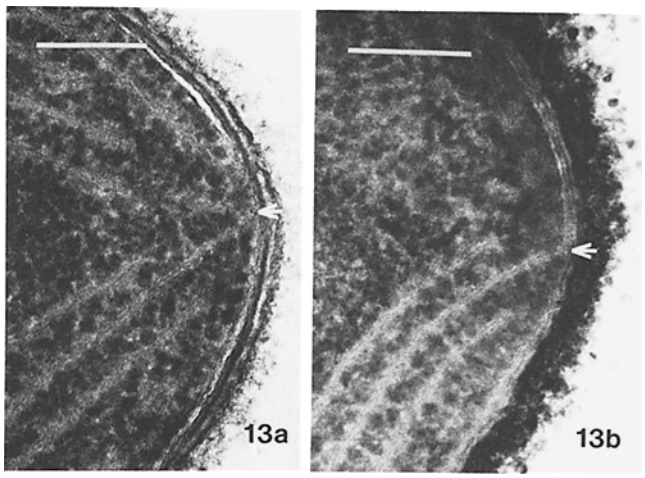

FIGURE 13 High-voltage electron micrograph of thick (0.25-μm) sections through *A. quadruplicatum* cells, illustrating contacts between thylakoid and cytoplasmic membranes (arrows). (a) Contact in cross section; note membranes tending to converge at a common point. (*b*) Contact in oblique section; manner in which thylakoids converge is distorted when viewed from oblique direction, but contact with cytoplasmic membrane remains visible in this case. Bars, 0.25 μm. × 64,000 (*a*); × 74,400 (*b*).

anobacterium. It should be realized that *Agmenellum quadruplicatum* was grown under carefully controlled conditions for this investigation and that only exponentially growing cultures were examined. The ultrastructural characteristics of this organism are known to vary under different growth conditions (21, 22). Therefore, further investigation would be required to understand fully the effects of cultural or other external factors on the three-dimensional arrangements described herein. It should also be realized that the three-dimensional ultrastructural characteristics of other cyanobacteria, especially the filamentous genera (1–3), probably differ from those of *A. quadruplicatum*. The other unicellular cyanobacteria may also differ from *A. quadruplicatum*, since they often appear to have distinct features in randomly cut thin sections (19, 37, 40–46). Complete three-dimensional analyses of other unicellular cyanobacteria would be needed to determine how universally the features found in *A. quadruplicatum* occur in this group of organisms.

One of the more interesting findings of the present study was the fact that the specialized inclusion bodies of *A. quadruplicatum* consistently occupied specific locations within the cell. For example, polyphosphate bodies and carboxysomes were always confined to the central cytoplasmic region of the cell (defined in Results). Micrographs of individual sections have implied that this arrangement occurs in other unicellular cyanobacteria (37, 40, 47, 48), but only the complete reconstructions carried out in the present study could demonstrate its existence conclusively. Direct contact between polyphosphate bodies and carboxysomes was seen occasionally and has been reported previously (29). The unordered clustering of these two inclusion bodies that was seen in three-dimensional reconstructions indicates that their occasional contact is more likely to occur by chance than as a result of any functional need. Similarly, the carboxysomes in *A. quadruplicatum* never appeared to be in direct contact with the thylakoid membranes in sections prepared with either fixation (see Materials and Methods). This is in contrast to the results of Peat and Whitton (49) who found that such contact always occurred in *Anabaenopsis* sp. Since carboxysomes may be directly involved in CO_2 fixation (34, 50) and since thylakoids are known to produce the reducing power required for CO_2 fixation, contact between these two structures to increase efficiency is not an unreasonable possibility. Nevertheless, the

FIGURE 11 Stereo pairs of CARTOS reconstructions in color, illustrating variety of features in complete cells. (a) Cell (outline in white dots) including most thylakoids (purple, yellow, green, blue, and red lines, starting with innermost membrane), carboxysomes (large white circles), and lipid bodies (small yellow circles). Cell is viewed along central longitudinal axis. Front pole of cell has been deleted to eliminate overlapping of thylakoids. Spacing between individual thylakoids is best seen along bottom edge of triangles in thylakoid pattern. Thylakoids taper toward tip at back of cell. (b) Side view of cell (outline in white dots) including two complete thylakoids (innermost in blue lines; outermost in green lines), carboxysomes (white circles), polyphosphate bodies (large, centrally located yellow circles), and lipid bodies (small, peripherally located yellow circles). Separation between the two thylakoids is best seen along bottom edge of cell. Tapering of thylakoids toward cell pole is especially clear at left. Note how thylakoids completely surround central cytoplasmic region of cell. Compare peripheral location of lipid bodies with central location of carboxysomes and polyphosphate bodies. (c) Different view of reconstruction shown in Fig. 11*b*; cell is viewed down its central longitudinal axis. Note triangular pattern formed by thylakoids in cylindrical portion of the cell. Part of the outermost thylakoid has been deleted to avoid excessive overlapping of details. (*d*) Side view of a dividing cell (outline in white dots) including two complete thylakoids, carboxysomes, polyphosphate bodies, and lipid bodies (all depicted as in Figs. 11, *b* and *c*). Thylakoids completely surround central cytoplasmic region in both halves of the cell and clearly taper to single points at both cell poles. Compare central location of densely clustered carboxysomes and polyphosphate bodies with peripheral location of lipid bodies.

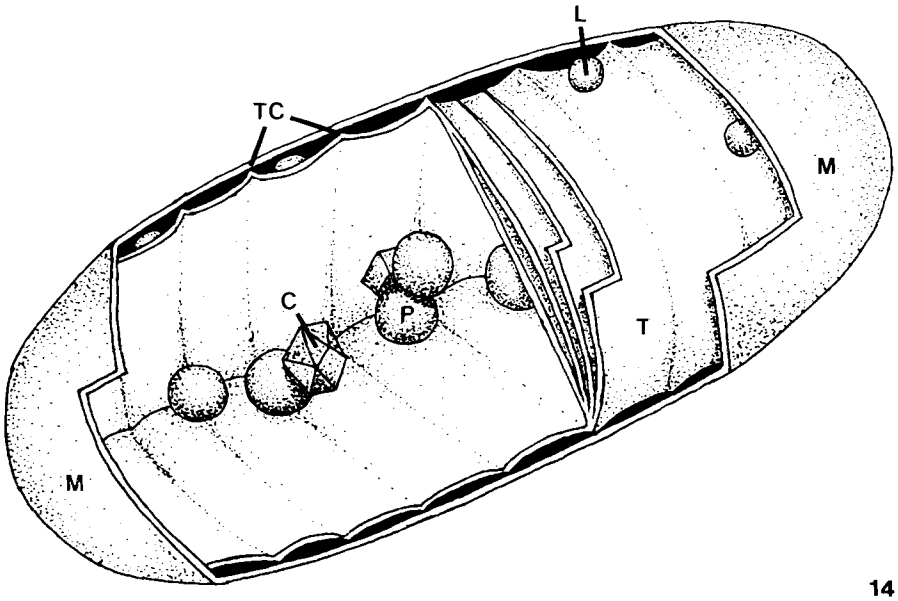

FIGURE 14 Artist's representation of the overall three-dimensional architecture of *A. quadruplicatum*. *C*, carboxysome; *L*, lipid body; M, cytoplasmic membrane; *P*, polyphosphate body; *T*, photosynthetic thylakoid membrane system; *TC*, contacts between thylakoid and cytoplasmic membrane. Thylakoids are depicted as solid sheets, each representing a pair of closely apposed unit membranes. The spacing between thylakoid-cytoplasmic membrane contact points is theoretical, as this was not determined precisely (see text). (Illustration does not include cell wall, ribosomes, and nuclear material.)

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present results have demonstrated clearly that contact is not required to maintain normal metabolic functions in rapidly growing cells.

The central cytoplasmic region of *A. quadruplicatum* was found to be a virtually independent compartment bounded by the innermost pair of thylakoid membranes. It may have had contact with the periphery of the cell at sites where thylakoid membranes merged and came in contact with the cytoplasmic membrane. The central cytoplasm and the surrounding thylakoid zone were separated from each other, however, as was indicated by the absolute and consistent segregation of inclusion bodies into one region or the other. This separation was also indicated by the results of previous studies on nutrient limitation (21, 22). In those studies, glycogen granules accumulated extensively throughout the thylakoid system but were not detected in the central cytoplasm until the thylakoid membranes began to deteriorate. The separation of the central cytoplasm from the rest of the cell in *A. quadruplicatum* appears to be an example of compartmentalization in a prokaryotic organism. It would be interesting to determine the functional advantage of compartmentalizing the cytoplasm in this way.

The detailed three-dimensional arrangement of the thylakoid membrane system in *A. quadruplicatum* was of major interest in this study because there has been considerable confusion over this topic in the literature. Most previous investigations have attempted to describe the three-dimensional arrangement of the thylakoid membranes in unicellular cyanobacteria by extrapolating from the appearance of these membranes in randomly cut, single sections. Several authors have described the thylakoids as a group of independent disks or flattened sacs (37, 43, 44, 51), while others have considered them to be truly concentric structures (2, 40, 41, 52–54). More recently, the thylakoid system has been described as "an anastomosing network of concentric shells" (55). The results of the present study have proven that, at least for *A. quadruplicatum*, the first two concepts cited above are incorrect. Our data are consistent with the concept of an anastomosing network, but further show for the first time the exact appearance of that network. The thylakoids of *A. quadruplicatum* clearly joined together, but only at or very near the cytoplasmic membrane. The individual thylakoids emanating from these peripheral intersections did not branch at other points in the cell. In contrast, branching of the thylakoids has been reported to occur in two other unicellular cyanobacteria, *Anacystis* and *Synechococcus* (37, 44, 45, 56).

The thylakoid network of *A. quadruplicatum* was found to be in contact with the cytoplasmic membrane at a number of locations in the cell. Contact between thylakoid and cytoplasmic membranes has been reported in a wide variety of cyanobacteria (40, 42, 45, 57–63) although reviewers generally consider this to occur only rarely (2, 55). Stanier and Cohen-Bazire (2) stated that the thylakoids and the cytoplasmic membrane are topologically distinct, even in cyanobacteria where one would expect to detect contact points easily if they were present. The contacts were quite difficult to find in the present study, especially when random single sections were viewed. Serial sections through complete cells were essential for detecting contacts between the thylakoid system and the cytoplasmic membrane routinely and for determining the locations of these contacts in the cell. The use of thick sections and HVEM also facilitated the discovery and location of contact points because the greater depth of material in these sections allowed one to understand better how the thylakoids were arranged with respect to the cytoplasmic membrane in three-dimensional space. The techniques used in the present study, then, might well detect previously unnoticed contacts between the thylakoids and the cytoplasmic membrane in other cyanobacteria.

The functional significance of contacts between thylakoids and the cytoplasmic membrane cannot be determined from the present results. It has been suggested that thylakoids arise by invagination of the cytoplasmic membrane (36, 40, 42, 45, 63, 64); thus, the contacts would most likely serve as sites for thylakoid synthesis. This suggestion might also explain why the lipid bodies, which could serve to store excess membrane components, were located only near the contacts. As Golecki and Drews (55) have pointed out, however, no one has demonstrated conclusively that thylakoids do originate from the cytoplasmic membrane. Similarly, the present study did not reveal definite evidence of lipid bilayer continuity between the thylakoid system and the cytoplasmic membrane. If the

contacts seen in *A. quadruplicatum* were not invaginations or centers for thylakoid synthesis, then their function may have been simply to maintain the three-dimensional arrangement of the thylakoid system. The merging of the thylakoids and their attachment to the cytoplasmic membrane allows the central cytoplasm to approach the periphery of the cell. This arrangement might facilitate segregation of nuclear material during cell division by providing sites for attachment of the genome to the cytoplasmic membrane, as is thought to occur in other bacteria. The thylakoid arrangement in *A. quadruplicatum* might also facilitate transport of substances in and out of the cytoplasm. If the thylakoids were arranged as independent and concentric cylinders without merging near the cytoplasmic membrane, then substances would have to cross several layers of semi-permeable membrane to enter or leave the central cytoplasm. Thus, the contacts in *A. quadruplicatum* may allow the central cytoplasm to have limited contact with the external medium while still being compartmentalized from the rest of the cell.

Although the arrangement of thylakoids in *A. quadruplicatum* was quite consistent from cell to cell, two variations that require explanation were observed. The first of these was the existence of an alternate cross sectional pattern in ~30% of the cells. Since no intermediate or transitional forms were observed and since the two patterns were not related to cell division, it is assumed that they represent an inherent variation in the culture. There may have been two distinct genotypes present, or the alternate thylakoid arrangement may have been coded by a plasmid. Further study will be required to determine the cause of this variation. The second variation was seen in cell poles and was clearly related to cell division. Since transitional forms between the "new" and "old" arrangements (Fig. 10) were detected, it is reasonable to assume that cell division left the thylakoid system in a slightly altered conformation that was later "corrected" by rearrangement of the membranes. A three-dimensional analysis of cell division would elucidate the details of this process, but was beyond the scope of the present study.

Perhaps the most interesting general aspect of *A. quadruplicatum*'s three-dimensional architecture is the fact that it was so consistent from one cell to another, even in the case of relatively complex or minute details. Such a high degree of intracellular organization within a prokaryotic organism must be the result of accurate control mechanisms or it would not be reproduced so consistently in each cell. We are currently investigating the mechanisms by which the precise three-dimensional arrangement of *A. quadruplicatum* is maintained.

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