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EXPERIMENTAL ARTICLES

Atypical Cell Forms Overproducing Extracellular Substances in Populations of Cycad Cyanobionts

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Abstract—The ultrastructure of the cyanobionts of the greenhouse-grown cycads *Cycas circinalis*, *Ceratozamia mexicana*, and *Encephalartos villosus* was studied. The cyanobiont microcolonies grown in the intercellular space of the cyanobacterial zone of cortical parenchyma in the cycad coralloid roots contained two specific forms of vegetative cells with a reduced cell wall, namely, protoplasts and spheroplasts. The protoplasts and spheroplasts exhibited ultrastructural properties indicating the overproduction of two extracellular substances, one of which resembled the mucilage polysaccharides and the other was protein-like. The substances were likely to be synthesized intracellularly and then be excreted with the aid of surface vesicles or by ruptures in the cytoplasmic membrane to form, respectively, a mucilagious extracellular matrix and an additional electron-opaque envelope around the cell. At the late developmental stages, the excretion of these substances was accompanied by degradative changes in the cells, leading eventually to cell death. The physiological role of these specific cell forms and the factors that induce their development and death in the cell populations of cyanobionts are discussed.

Key words: symbiosis, cycads, ultrastructure, cyanobionts, protoplasts, spheroplasts, intercellular matrix, mucilage, polysaccharides.

Symbiotic cyanobacteria are an interesting object for studying the structural, functional, and morphological diversity of prokaryotes living in macrosymbiont cells and tissues. As compared to free-living cyanobacteria, the cyanobionts of plants are characterized by an enhanced fixation of atmospheric nitrogen, a decreased or totally suppressed activity of photosynthesis and cell multiplication, an altered cell morphology, and the predominance of single, large cells [1]. The cyanobionts of cycad plants [2, 3] and the liverwort *Blasia pusilla* [4] showed still more profound structural changes in the cell wall, giving rise to cell forms with a reduced cell wall, such as protoplasts and spheroplasts. The morphological modification of cyanobionts indicates that not only nitrogen fixation but also other important physiological processes in cyanobacteria undergo changes under symbiotic conditions. The ultrastructure of cells is an important indicator of their physiological state, which can also give some information on the possible causes and mechanisms of the structural and functional variability of the cells.

Cyanobacteria are known to be able to symbiose with taxonomically very diverse higher plants, namely, bryophytes (liverworts and hornworts), the mosquito ferns of the genus *Azolla*, the gymnospermous plants of the family *Cycadaceae*, and the angiospermous plants of the genus *Gunnera*. Different symbiotic associations exhibit some differences in the functioning and structural organization of symbiotic zones.

The aim of this work was to study the ultrastructure of the cyanobionts of the poorly studied cycads *Encephalartos villosus* Lehm. and *Cycas circinalis* L. and the hitherto unstudied cycad *Ceratozamia mexicana* Brough.

Cycadaceae is one of three families of the order *Cycadales* of the class *Cycadopsida*. The order includes about 150 living species of relict gymnospermous plants. All the cycads that have been studied in relation to symbiosis were found to be able to symbiose with cyanobacteria [1]. The ultrastructure of the cyanobionts coexisting with cycad plants has been most thoroughly described by Grilli Caiola [2].

MATERIALS AND METHODS

Investigations were carried out with fragments of the apogeotropic roots of the *Cycas circinalis* L., *Ceratozamia mexicana* Brough., and *Encephalartos villosus* Lehm. plants grown in greenhouses with subtropical and tropical climates in the Tsitsin Central Botanical Garden of the Russian Academy of Sciences. The *E. villosus* plants were 30- and 50-years-old, and the *C. circinalis* and *C. mexicana* plants were 50-years-old. Samples of the apogeotropic roots of these plants were collected in January, April, and June of 2001. Root fragments were cut from the apical region (1–3 mm

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from the apex), middle region (4–10 mm from the apex), and basal region of the apogeotropic roots.

The longitudinal and radial sections (10–30 μm thick) of the apogeotropic roots were stained with an aqueous solution of ruthenium red or a solution of this dye dissolved in cacodylate buffer (pH 6.8) containing 1% glutaraldehyde. Some sections were preliminarily fixed by the Cornua method. Specimens were analyzed under a Laborlux D (Leitz) light microscope.

Specimens for electron microscopic studies were fixed with 2% glutaraldehyde in cacodylate buffer for 30 min, refixed with 1% OsO_4 in the same buffer for 4 h, and dehydrated in a series of alcohol solutions of increasing concentration (up to absolute alcohol saturated with uranyl acetate). The preparation was then embedded in araldite epoxy resin. After curing, thin sections were cut on an LKB-4800 ultratome (Sweden), contrasted with lead citrate[2] or uranyl acetate (either 2% aqueous solution or 25% solution prepared on absolute methanol), and examined in a JEM-100B electron microscope.

For spectroscopic studies, cyanobacterial cells recovered from root fragments were suspended in distilled water, and the absorption spectra of this suspension were recorded using a Hitachi 150-20 spectrophotometer equipped with an optical sphere harvesting the scattered light. The spectra were corrected for light scattering as described by Merzlyak and Naqvi [5].

RESULTS AND DISCUSSION

The root system of cycad plants is characterized by the development of specific, dichotomously branched, apogeotropic roots (called also coralloid roots or coralloids). The coralloid roots develop from the secondary lateral roots of cycad plants and contain symbiotic cyanobacteria, which colonize the coralloid roots in the process of their development. The symbiotic cyanobacteria, which mainly belong to the genus *Nostoc*, are primarily located in the intercellular space of cortical parenchyma. The cortical parenchyma colonized by symbiotic cyanobacteria is characterized by the development of a specific slime zone in the form of a cylinder extending along the entire coralloid root except for its apex. This zone, in which symbiotic cyanobacteria live, is called the cyanobacterial zone.

The analysis of the coralloid root samples showed that the cyanobiont population grew in the intercellular space of the cyanobacterial zone of cortical parenchyma, forming microcolonies. In the case of *C. circinalis*, symbiotic cyanobacteria might also occur individually in degrading plant cells. The microcolonies were found to contain cyanobacterial filaments, single vegetative cells, heterocysts, and ultrastructurally diverse vegetative cells with a defective cell wall (VCDCW). The alteration or the profound degradation of the peptidoglycan layer led to the formation of spheroplasts, whereas the concomitant reduction of the outer membrane led to the formation of protoplasts. Irrespective of the type of cells, they were embedded in the intercellular matrix, whose ultrastructure resembled that of the gelling polysaccharides of mucilage (Fig. 1). The microscopic examination of the ruthenium red–stained sections of the coralloid roots of *E. villosus* and *C. mexicana* showed that the intercellular matrix stained pink, indicating the presence of acidic polysaccharides in the matrix. The thin fibrils and fine granules of the matrix became visible on thin sections after contrasting them with either lead citrate or uranyl acetate. Both methods of contrasting also revealed a fibrillar structure of the middle lamella, which is made of the acidic polysaccharide pectin. The slimy sheaths of vegetative cells, which are typical structures of the *Nostoc* cyanobacteria, were not revealed. At the same time, VCDCWs were detected in all three regions (apical, middle, and basal) of the coralloid roots. It should be noted that the problem of the appearance of cell forms with the defective cell wall and the occurrence of L transformation processes in the cyanobiont populations were discussed in our earlier work [6]; however, the coralloid roots of *C. circinalis*, *C. mexicana*, and *E. villosus* contain protoplasts and spheroplasts, i.e., vegetatives cells with a reduced cell wall (VCRCW), which differ ultrastructurally from the L-like forms of cycad cyanobionts.

VCRCWs of the first type were primarily represented by protoplasts with ultrastructural properties indicating an intense production and secretion of a substance resembling in ultrastructure the mucilage filling the intercellular space. The microscopic examination of the root samples taken in June showed that such VCRCWs were located near intact vegetative trichomes occurring, for instance, in the apical region of the coralloid roots of *C. circinalis* (Fig. 1). Cyanobacterial cells in the trichomes differed in shape but had the same ultrastructure as the free-living cyanobacteria cultivated under optimal growth conditions in the light. This suggested that the cyanobacterial cells could actively grow in the trichomes. At the same time, contiguous VCRCWs, including protoplasts, exhibited an entirely different structure. Ribosomes and nucleoid zones typical of cyanobacteria were not visible on the thin sections contrasted with lead citrate or uranyl acetate. Most of the cytoplasm contained the depositions of a fine granular and fibrillar substance packaged reticularly, much in the same way as the mucilagious extracellular matrix was, except that the matrix package was looser (Fig. 2). The fibrils occurring in the cytoplasm and the intercellular matrix had a thickness of about 5 nm. The fibrillar material filled the space between the thylakoids and the cytoplasmic membrane (CM). The dense depositions of the fine granular and fibrillar substance were mainly observed in the interthylakoid space, often being located very close to the external

[2] Presented are only the micrographs of specimens contrasted with lead citrate.

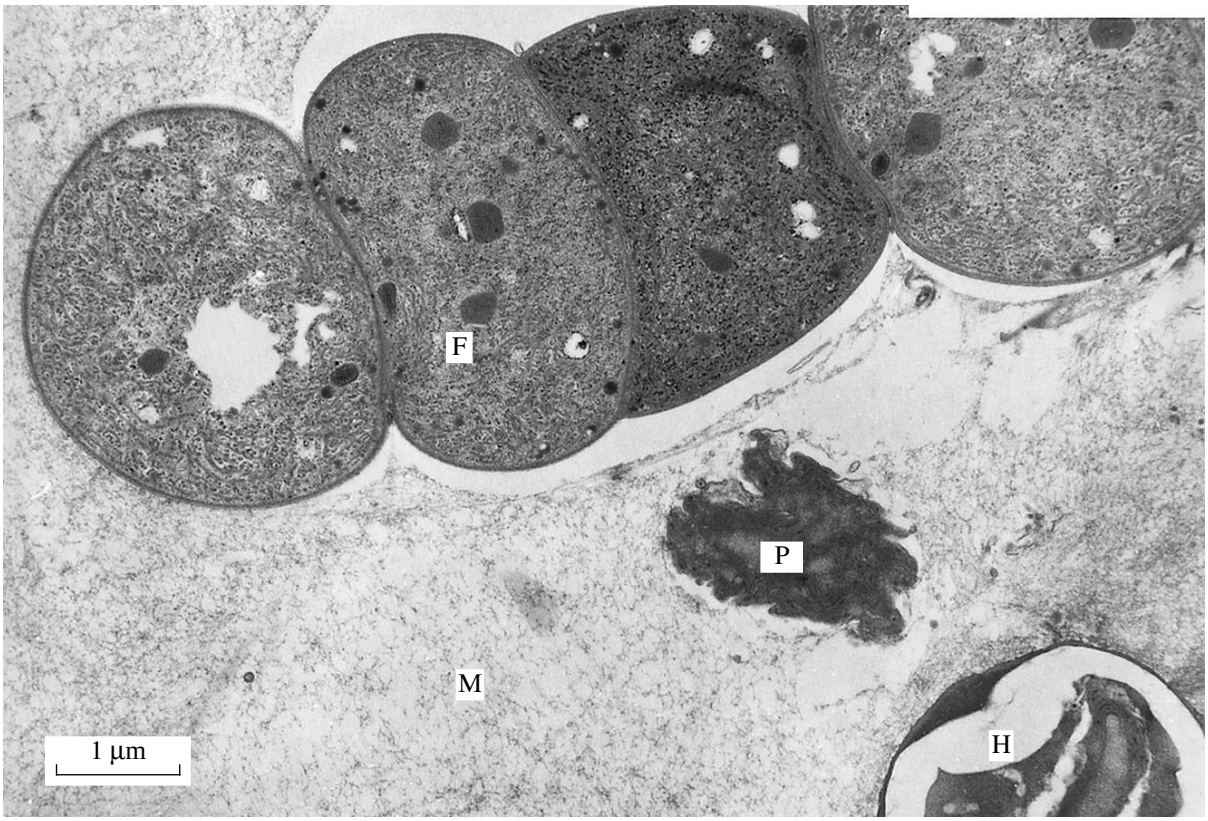

Fig. 1. A cyanobiont microcolony in the intercellular space of the cyanobacterial zone of cortical parenchyma near the coralloid root apex of *C. circinalis.* F, filament composed of intact vegetative cells; P, protoplast with ultrastructural properties indicating the overproduction of a substance similar to polysaccharides of the mucilage; H, ultrastructurally altered heterocyst; M, mucilaginous intercellular matrix.

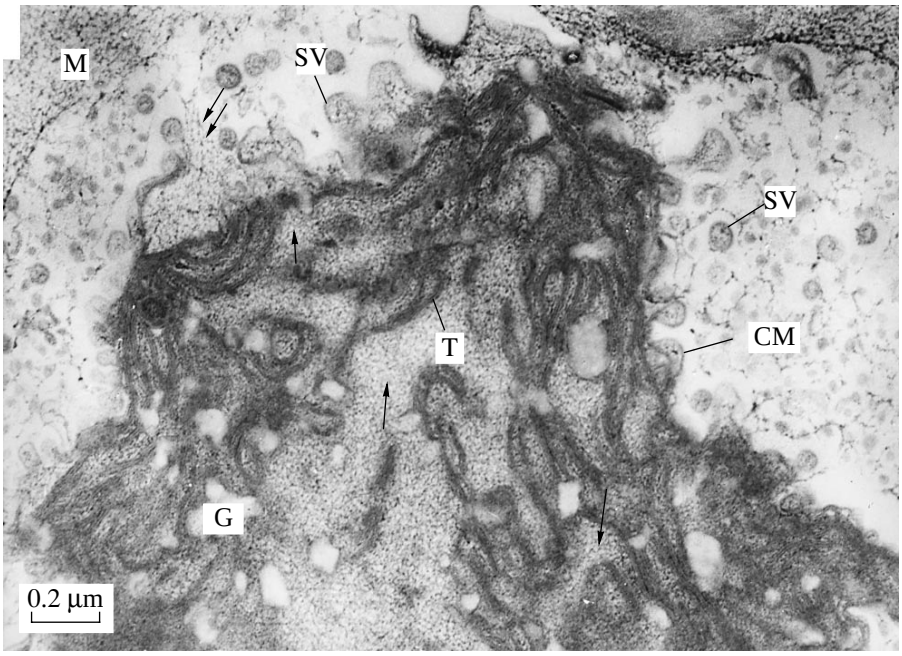

Fig. 2. A cyanobiont protoplast in the basal region of the coralloid root of *E. villosus.* One arrow shows the depositions of a fine granular and fibrillar substance in the protoplast cytoplasm and two arrows show the site where this substance is released by ruptures in the CM. T, thylakoid; G, unidentified granule; CM, cytoplasmic membrane; SV, surface vesicle. The other designations are as in the legend to Fig. 1.

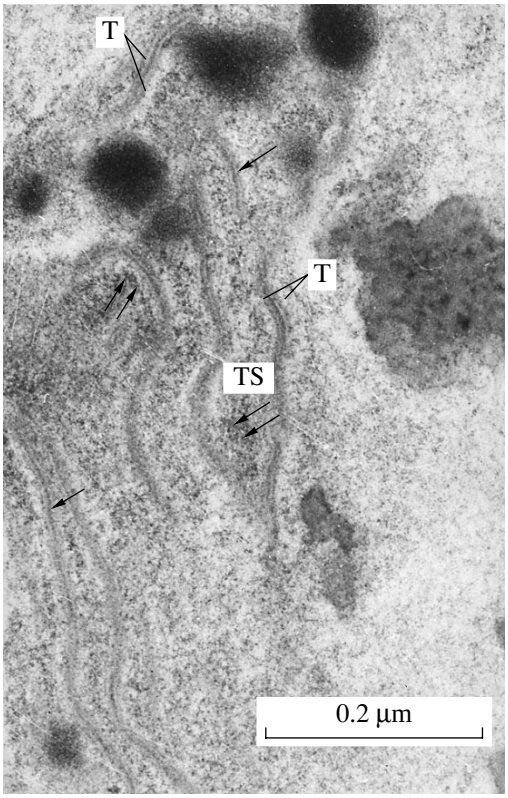

Fig. 3. A fragment of a cyanobiont protoplast in the middle region of the coralloid root of *C. mexicana.* One arrow shows a thylakoid interior with the closely pressed thylakoid membranes, and two arrows show the depositions of a dense substance. T, thylakoid; TS, interthylakoid space.

surface (facing to the cytoplasm) of the thylakoid membrane (Fig. 3). No phycobilisomes in these cell forms were detected. Thylakoid membranes with distinct internal surfaces were close to each other. It should be noted in this regard that the method of thin section fixation used in this work allowed the thylakoids to be more clearly seen than did the same fixation method in an earlier comparative study of symbiotic and free-living cyanobacteria.

The inner thylakoid space was not enlarged. Some adjacent thylakoids were closely located to form stacks. The absorption spectra of the freshly isolated thylakoids of the cyanobionts of all of the cycad plants under study had the peaks of chlorophyll (440 and 675 nm), phycocyanin (630 nm), and carotenoids (shoulders at 440 and 490 nm) typical of cyanobacteria (Fig. 4 shows the absorption spectrum of the thylakoids of the cyanobiont of *C. mexicana*). It should be noted that the proportions between the absorption peaks of the photosynthetic pigments corresponded to actively growing free-living cyanobacteria [5].

The mucilage-like cytoplasmic material located between the peripheral thylakoids and the CM was ingulfed into the membrane vesicles pinched off into the intercellular matrix (Fig. 5). In some protoplasts, the vesicles were observed on the entire surface. Some vesicles were located near the VCRCW surface (Figs. 2, 5). These data suggest that the VCRCWs may actively synthesize the mucilage-like substance and release it by the mechanism of the CM-mediated vesicular transport.

Some protoplasts of cyanobionts in the coralloid roots of *C. mexicana* and *E. villosus* contained very large and irregularly shaped cyanophycin granules (Fig. 6). In the case of the cyanobiont of *E. villosus*, the protoplasts also contained numerous homogeneous granules which appear electron-translucent on microphotographs (Figs. 2, 5). The granules may protrude, together with the CM, beyond the cell surface (Fig. 5).

VCRCW may differ somewhat structurally from each other. Some VCRCWs were large, round, and contained thylakoids with a different shape. Such VCRCWs were often detected in the cyanobionts living in the apical region of the coralloid roots of *C. mexicana* sampled in June (in the root samples taken in April, such VCRCWs were also detected but rarely). Vast undulating regions of the cell surface often contained not one but two membranes separated by a layer of a substance having the same structure as in the cytoplasm (Fig. 7). The outer membrane of these VCRCWs did not exhibit any ultrastructural features distinguishing it from the CM. Surface vesicles with a substance resembling that of the cytoplasm could be formed in these regions with the involvement of the outer membrane. Some of these VCRCW could be identified as spheroplasts with an irregularly enlarged periplasmic space. The thylakoids of such cell forms were shortened and contained dense depositions, which were more abundant than in the protoplasts presented in Figs. 2, 3. The substance ultrastructurally similar to the cytoplasmic material was also detected in a local region beyond the outer membrane (Fig. 7).

The fine granular and fibrillar substance filling the interior of VCRCW and surface vesicles is likely to represent acidic polysaccharides, as can be suggested from the similarity of its ultrastructure to that of mucilage occurring in the cyanobacterial zone of coralloids (Figs. 2, 5–7), which is known to contain acidic polysaccharides. This suggestion is supported by the observation that the degradation of the VCRCWs of this group is accompanied by the formation of ruptures in the CM, by which the cytoplasm is linked with the intercellular matrix by means of specific fibrillar strands (Figs. 2, 8). The outer membrane present in some VCRCWs can break, making possible the fusion of the intra- and extracellular substances (Fig. 9). These ultrastructural changes in the VCRCWs indicate that these cell forms overproduce a substance that is involved in the formation of the mucilagious intercellular matrix in the cyanobacterial zone of the coralloid roots. At the late stages of VCRCW development, this process is accompanied by the degradation of the reproductive and protein-synthesizing systems and the impairment of the CM, eventually causing the death of the VCRCW. The CM under-

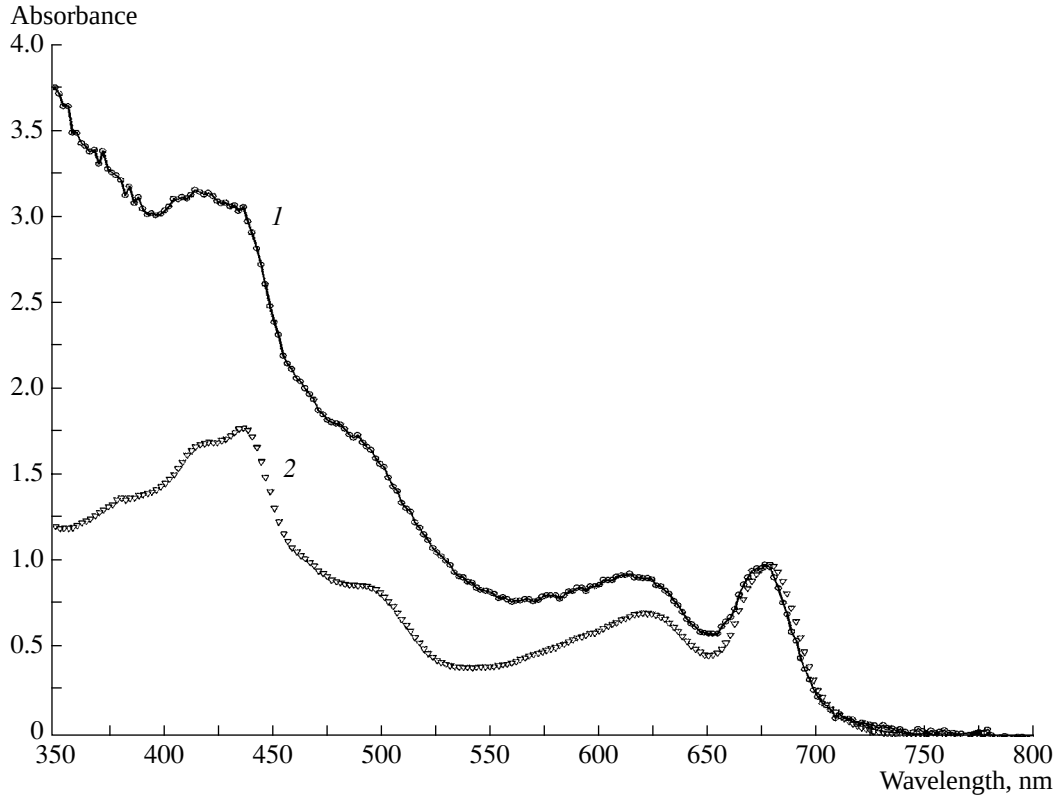

Fig. 4. The spectra (normalized at 680 nm) of the cyanobionts isolated from the coralloids of *C. mexicana* in (*1*) April and (*2*) June 2001.

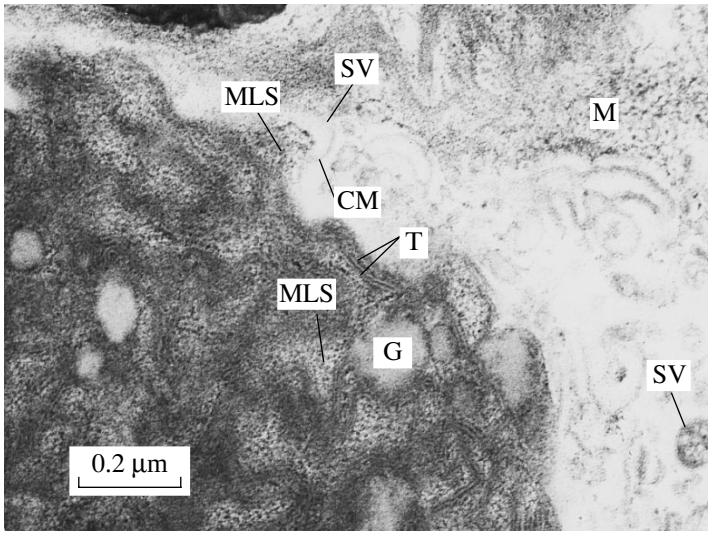

Fig. 5. A fragment of a cyanobiont protoplast in the basal region of the *E. villosus* coralloid. MLS, mucilage-like substance. The other designations are as in the legends to Figs. 1 and 2.

goes complete lysis, the cytoplasm merges with the extracellular substance, and the thylakoids prove to be embedded in the micilagious matrix (Fig. 10). These destructive processes do not affect the structure and the atypical shape of the cyanophycin granules.

These degradative changes in the VCRCW are analogous to those observed in the mucilage-producing cells of the root cap [7]. In both cases, cells excrete intracellularly synthesized substances with the aid of vesicles, then undergoing progressive degradation and

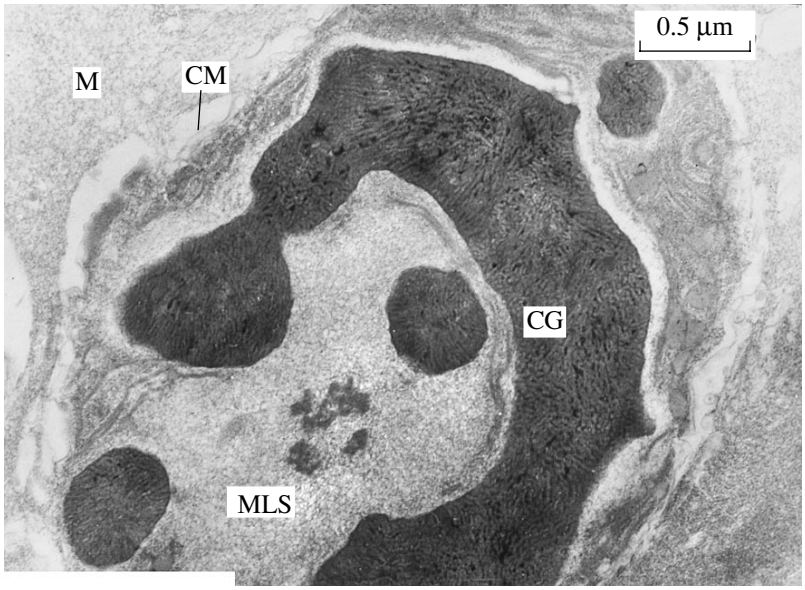

Fig. 6. A cyanobiont protoplast in the middle part of the *C. mexicana* coralloid. CG, cyanophycin granule. The other designations are as in the legends to Figs. 1, 2, and 5.

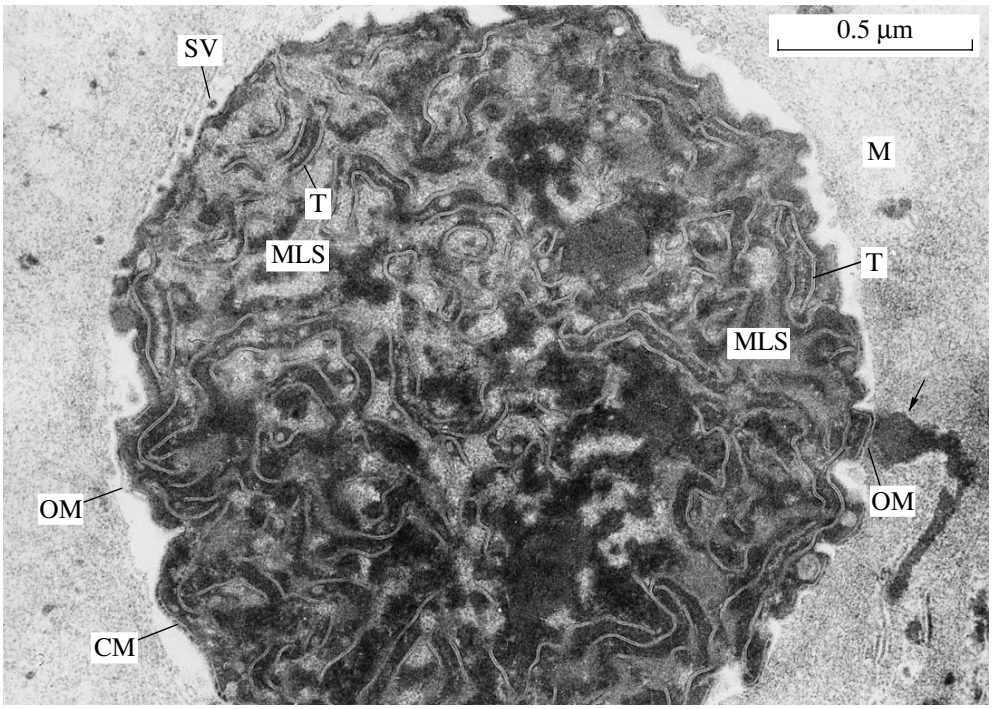

Fig. 7. A VCRCW of the cyanobiont in the apical region of the *C. mexicana* coralloid. The arrow shows the deposition of the dense substance. OM, outer membrane. The other designations are as in the legends to the previous figures.

becoming eventually part of the mucilaginous matrix. The most degraded VCRCWs were observed in the basal zones of the coralloid roots.

Thus, the data obtained indicate that the cyanobiont population contains cell forms specialized in the production of a substance resembling ultrastructurally the substance of the intercellular matrix, which is likely to be an acidic polysaccharide. The possibility that these specific cell forms belong to a different strain should obviously be excluded, since such cell forms, though being connected to intact vegetative cells, were observed by us at the stage of the murein layer degrada-

tion. It is likely that there is only one mechanism of VCRCW formation, while the subsequent differentiation of VCRCWs is explained by their different specializations, for instance, as persistent L forms or producers of the mucilaginous matrix. This phenomenon is typical of different species and genera of cycad plants and, hence, may refer to different strains and even species of symbiotic cyanobacteria.

The intercellular space of the cortical parenchyma of the *C. circinalis* coralloids contained not only the mucilage-producing VCRCWs but also other types of VCRCWs (both protoplasts and spheroplasts) with the ultrastructural changes indicating the intense synthesis and excretion of proteins (Figs. 11, 12). These cell forms had nucleoid zones and clusters of regularly arranged ribosomes which were larger and more clearly seen than in the typical vegetative cyanobacterial cells. Among the ribosome clusters located on the periphery of the nucleoid zone were electron-opaque fibrils (Fig. 12) of a material that could be identified as protein-like based on its cellular location and electron density. Cyanophycin granules, which represent the depositions of the reserve nitrogen source of cyanobacteria (a polypeptide containing arginine and aspartic acid), were not detected. This observation is consistent with the ultrastructural manifestations of enhanced protein synthesis (such as enlarged nucleoid and a considerably increased number of ribosomes in the cell) observed by us in this study and by Gorelova in a model association of the cyanobacterium *Nostoc muscorum* CALU 304 with the rauwolfia callus [8]. In spite of the enhanced biosynthetic activity and the intact structure of various cytoplasmic components, including thylakoids, some VCRCWs had rupture in their CM, by which the protein-like substance could be released into the intercellular space (Fig. 12). Around the intact cells and the VCRCWs of the cyanobiont, there were electron-opaque envelopes and/or conglomerates of a fibrillar substance (Fig. 11). The cytoplasm of some degraded cells was filled with an electron-opaque fibrillar substance, whereas ribosomes, nucleoid, and thylakoids were not detected (Fig. 13). After these cells had been completely degraded, their contents were merged with the intercellular matrix, as in the case of the mucilage-producing VCRCWs described above. Figure 13 shows a local region where the protein-like cytoplasmic substance releasing by the CM rupture merge with the electron-opaque envelope surrounding the VCRCW.

Thus, the VCRCWs of this type display the ultrastructural changes indicating the synthesis of the protein-like extracellular substance. The ultrastructure of these VCRCWs considerably differs from that of the VCRCWs specialized in the synthesis of the mucilage-like substance. The existence of these two cell forms shows how variable the ultrastructure of symbiotic cyanobacteria can be. Moreover, we also observed ultrastructural changes in the cyanobiont heterocysts, which were similar to the described ultrastructural changes in the vegetative cells of cyanobionts. The latter phenomenon is the subject of our accompanying paper.

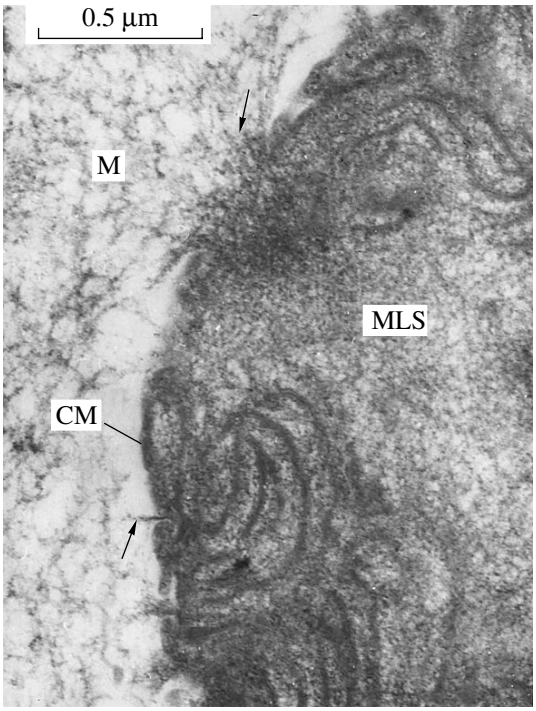

Fig. 8. A fragment of a cyanobiont protoplast in the apical region of the *C. mexicana* coralloid. The arrows show fibrillar strands linking the intra- and extracellular substances. Designations are as in the legends to the previous figures.

To the best of our knowledge, the accumulation in the cytoplasm and the subsequent excretion of substances resembling the polysaccharides of the intercellular matrix and the proteins of the extracellular envelope have not so far been described in the literature either for cyanobacteria or for other bacteria. The excretion of great amounts of slime by the filamentous gliding cyanobacteria of the order *Oscillatoriaceae* during their motion was found to occur through specific pores in the cell wall [9], which play the same role as the prokaryotic organelles involved in the rapid secretion of extracellular carbohydrates.

The VCRCWs that synthesize the substance resembling the polysaccharides of the intercellular matrix contain two membrane structures, thylakoids and the CM. The cellular location of this substance, which is often electron-opaque, near the external surface of the thylakoid membranes suggests that they may be involved in the synthesis and polymerization of polysaccharide macromolecules. It is known that some membrane-associated enzymes and carriers take part in the biosynthesis of the exopolysaccharides and the components of the bacterial cell wall from intracellular substrates, such as sugar mucleotides [10]. Some relevant problems, including the spatial location and the sources of necessary substrates, remain obscure. The possibility of their location in the inner thylakoid space

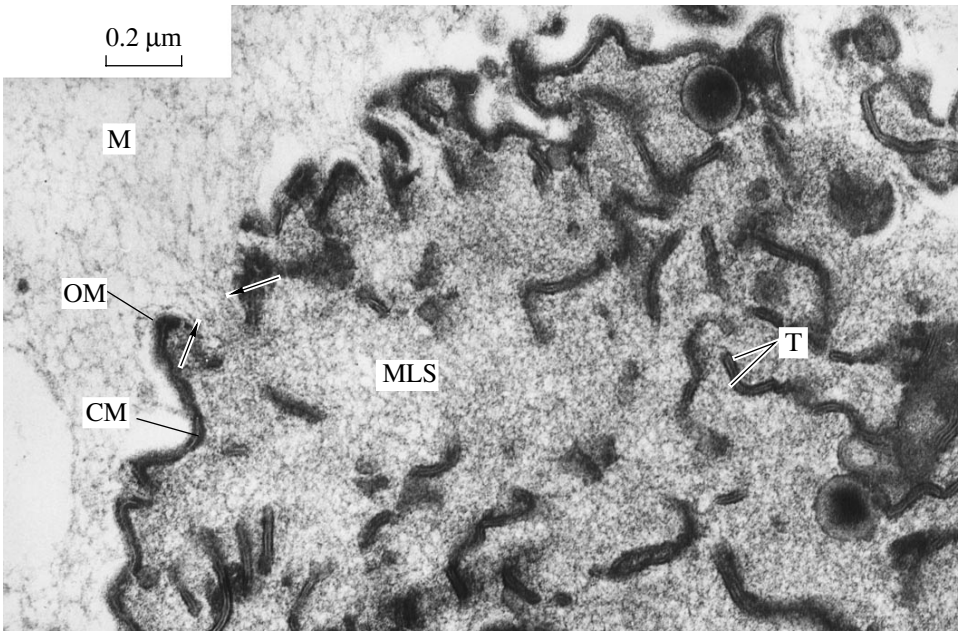

Fig. 9. A fragment of a cyanobiont VCRCW in the apical region of the *C. mexicana* coralloid. The arrows show fibrillar strands linking the intra- and extracellular substances. Designations as in the legends to the previous figures.

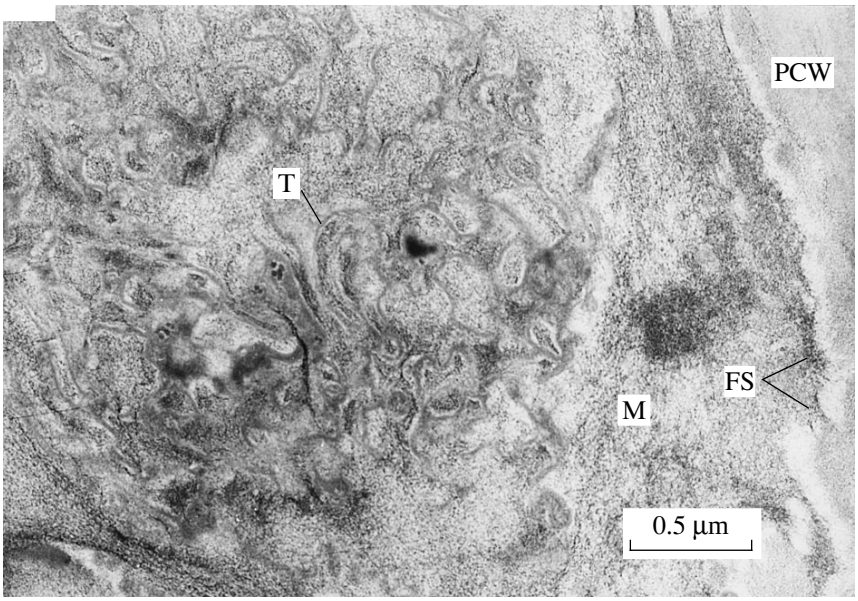

Fig. 10. A fragment of the intercellular space in the cyanobacterial zone of cortical parenchyma near the coralloid root apex of *E. villosus*. PCW, plant cell wall; FS, fibrillar strand of the intercellular mucilage. The other designations as in the legends to the previous figures.

and transport across the thylakoid membrane should obviously be excluded, since the thylakoid membranes were found to be closely pressed together in all of the VCRCWs analyzed. As for the substrate sources, the observed partial degradation of ribosomes and the nucleoid suggests that nucleotides may serve as such substrates. The necessary sugars may come from the degradation of the starch of the plant cells of the cortical parenchyma in the zone colonized by the symbiotic cyanobacteria. At the same time, the absence of any visible contact between the extracellular fibrils of the slimy matrix and the external surface of the CM suggests that the CM is unlikely to be involved in the biosynthesis of carbohydrate chains by the known mecha-

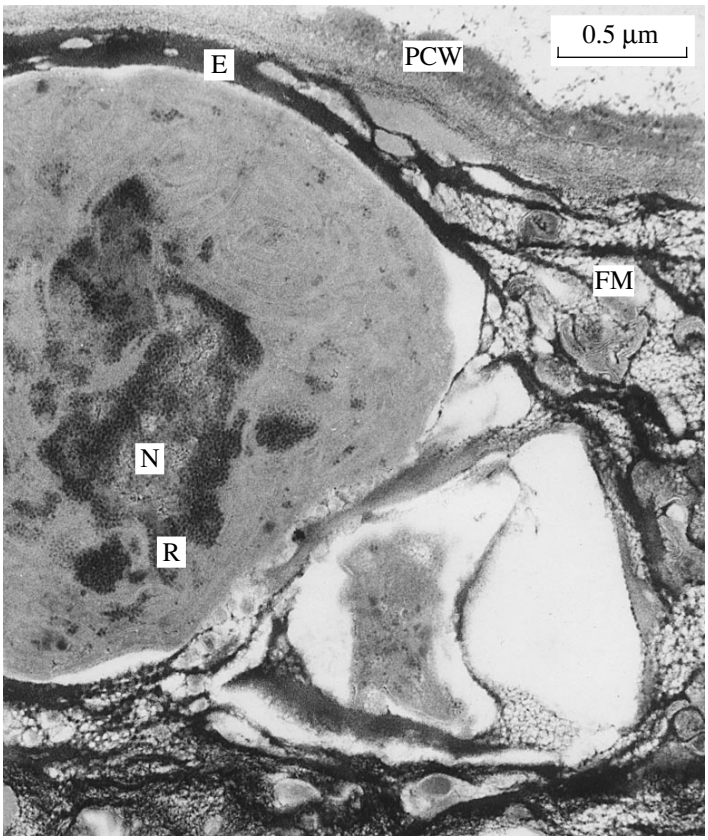

Fig. 11. A fragment of the intercellular space in the cyanobacterial zone of cortical parenchyma near the coralloid root apex of *C. circinalis* with a cyanobiont VCRCW overproducing a protein-like substance. FM, electron-opaque fibrillar material; E, electron-opaque envelope; N, nucleoid; R, ribosome. The other designations are as in the legends to the previous figures.

nisms associated with the transmembrane transfer of precursors (at least at the stage of their intense excretion studied in this work). The mucilage-like substance binds as a rule closely to the internal surface of the CM, and releases from the cells with the aid of the surface membrane vesicles or, in the case of profound cell degradation, by ruptures in the CM. The occurrence of the vesicles filled with the intracellular substance indicates that it is probably synthesized inside the VCRCW. The synthesized substance is excreted from the protoplast in much the same way as occurs in the Golgi apparatus, except that the compartmentation of polysaccharide synthesis in the VCRCW is likely to have some specific features. This problem needs further study.

The mucilage–like substance synthesized by the VCRCWs did not form sheaths around them but filled the entire intercellular space more or less uniformly. Near the plant cell wall, this substance occurred as fibrillar strands attached to the internal surface of the wall (Fig. 10). The package of fibrils in the strands differed from that observed on the periphery of the plant cell wall. The plant cells themselves exhibited no manifestations of the active production of mucilage. In particular, they did not have the developed Golgi apparatus, exocytosis-specific vesicles, and slimy depositions between the plasmalemma and the cell wall. This observation is consistent with the presence of specific mucilage-producing cell forms in the cyanobiont population of cycad plants. It can be suggested that mucilage in the intercellular space of cycads plants is of cyanobacterial origin, at least partially. This suggestion is supported by the data of Ahern and Staff [11], who showed that the intercellular mucilage in the coralloid roots of the *Macrozamia communis* seedlings appeared only after they had been infected with cyanobacteria. Furthermore, the mucilage was localized near the symbiotic cyanobacterial cells. Our 3-year observations of mature cyanobacterium-free *C. circinalis* plants also showed that the coralloid roots of these plants did not contain mucilage (in these experiments, more than 100 samples of the coralloid roots were analyzed).

Noteworthy is the fact that the profound rearrangement of cyanobacterial cells aimed at the overproduction of the substance resembling polysaccharides of intracellular matrix was accompanied by a reduction and then a loss of the nucleoid and other cellular structures (except for the thylakoids and cyanophycin granules), culminating in cell death. This suggests the triggering of the mechanism of apoptosis (programmed cell death) at a certain stage of cell rearrangement.

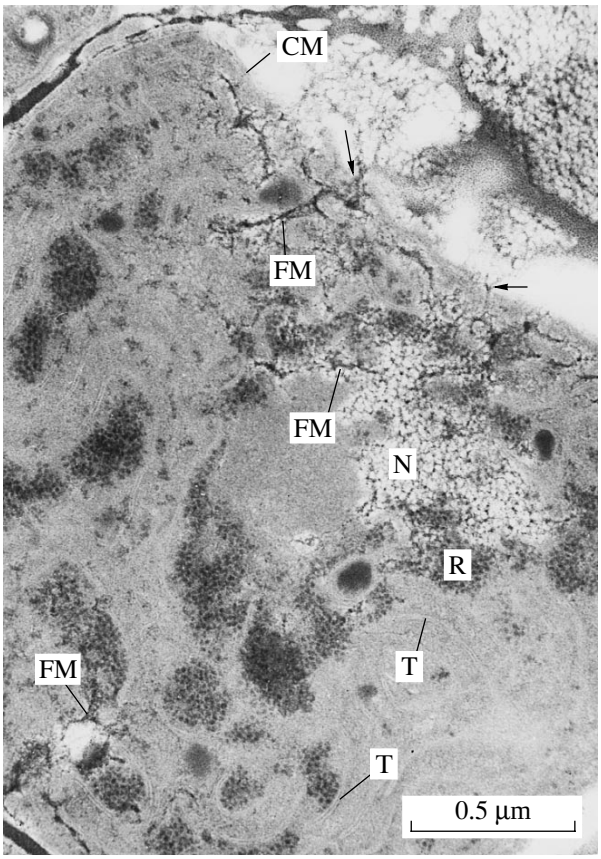

Fig. 12. A fragment of a cyanobiont protoplast near the coralloid root apex of *C. circinalis.* The arrows show the sites where the protein-like substance is released into the extracellular space. Designations are as in the legends to the previous figures.

In eukaryotes, apoptosis is triggered by the induction of the expression of caspases, whose cascade action leads to a rapid degradation of various structural cell components, including the nuclear membrane and DNA [12]. The most striking example of apoptosis in prokaryotes is the programmed death of part of the population of myxobacteria at the stage of their life cycle corresponding to the formation of fruit bodies [13], dead cells serving as a substrate for the production of the intercellular matrix of the fruiting bodies. Another example of sacrificial (in other words, programmed) death is the death of necridial cells in the trichomes of the *Phormidium* cyanobacteria, which serves as a mechanism of trichome separation into segments [14]. However, necridial cells do not produce mucilage and do not undergo specific structural rearrangement.

The production of extracellular polymers (predominantly polysaccharides) and the formation of an intercellular matrix is a specific property of prokaryotes living as colonies and/or biofilms. The intercellular matrix of pathogenic bacteria is a structural element of their colonies, which provides for the normal functioning of populations representing the polymorphic multicellular systems of bacteria, including those with the defective cell wall (i.e., VCDCWs) [15]. Similar concepts have been developed for the structural and functional organization of biofilms as specific microbial populations [16]. The study of the symbiotic bacteria of the genus *Nostoc* grown as microcolonies in the specific slime cavities of the *Blasia pusilla* thallus showed an important role of the intercellular matrix in the structural organization of the microcolonies and in the association of cyanobacterial cells, protoplasts, heterocysts, and microcells into a cyanobiont population [4]. Gorelova et al. suggested that the chemical nature of the gelling extracellular polymeric substances of the cyanobacterial sheath and the colonial matrix is similar to that of the animal extracellular matrix and, hence, that these substances may play the same role they play in animals, i.e., to provide for the intercellular transport of metabolites and its regulation.

The production of mucilage by the cycad cyanobionts may be a protective response of symbiotic cyanobacteria to the action of the phenolic compounds produced by parenchymal cells in the intercellular space where these cyanobacteria live [3]. It should be noted in this regard that bacterial cells in biofilms are more resistant to antimicrobial agents and disinfectants than the same bacteria living freely [16].

The compound processes leading to the formation of mucilage-producing VCRCWs may be aimed at protecting the cyanobiont population from the bactericidal agents (phenolic compounds) produced by the host plant. It is beyond doubt that any adaptive rearrangement in bacterial cells occurs in response to the action of some external physical or chemical factors. Bacteria are able to adapt to variable environmental conditions by varying their behavior, which is controlled at different levels, including the level of gene expression. In response to the action of various external factors, motile cyanobacteria can change the type of exopolysaccharides produced [17]. In natural and artificial symbioses, cyanobacteria undergo the action of the host plant, which may be either physical (such as a limited volume of the habitat provided by the host plant to the symbiotic cyanobacterium) or chemical (the action of various extracellular agents produced by the host plant) [1, 6]. In response, the symbiotic cyanobacterium produces heteromorphic cells with a partially or completely reduced peptidoglycan layer [8], the production of such cells probably being an adaptive response of the microsymbiont [6, 8]. In recent years, a concept has been developed that the growth and behavior of microbial populations are governed by the cooperative interactions of microbial cells, coordinated by the cell-to-cell communication signals [18]. Many bacteria are able to regulate the gene expression of the so-called quorum-sensing signals, whose generation depends on the cell density in a population. Such signals were found to regulate many properties of bacterial cells in the *Pseudomonas aeruginosa* biofilms [16]. In cyanobacteria, the cell-to-cell signal communication

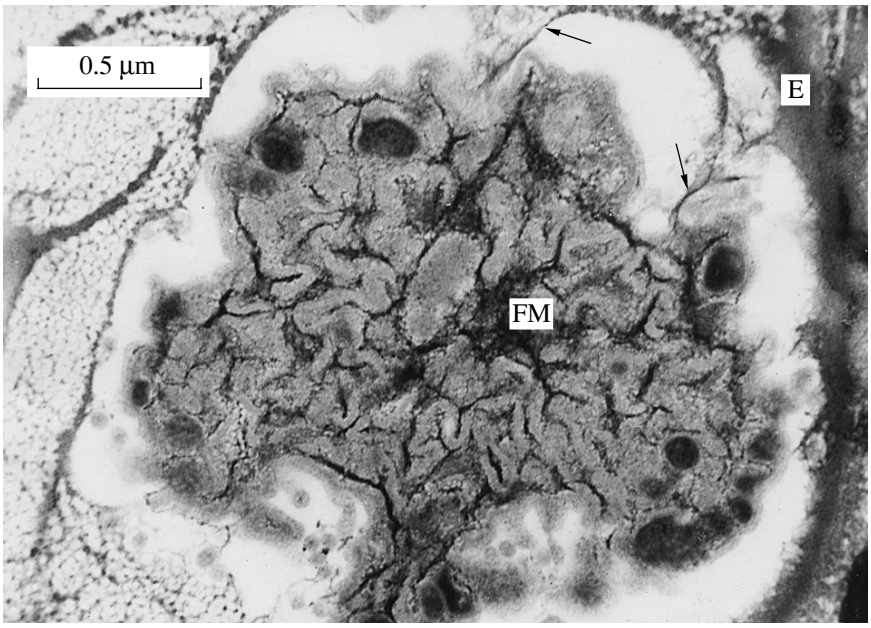

Fig. 13. A degrading VCRCW of the cyanobiont in the intercellular space of the cyanobacterial zone of cortical parenchyma near the coralloid root apex of *C. circinalis*. The arrows show the sites where the electron-opaque material of the cytoplasm merges with the electron-opaque VCRCW envelope. Designations are as in the legends to the previous figures.

may be due to the cyanobacterial toxin microcystin [19]. The coordinated behavior of bacterial cells may arise as they modify their environment through the consumption of nutrients, the discharge of toxic metabolites and some enzymes, and the formation of an extracellular matrix (such as a capsule) [20].

There are no grounds to deny the possibility of the existence of intrapopulation signals and the cooperative behavior of cyanobacterial cells in symbiotic microcolonies. First, the similarity between the architectonics of the cyanobiont microcolonies and biofilms suggests the existence of the cell-to-cell interactions mediated by quorum-sensing signals. Second, the VCRCWs specialized in the overproduction of the mucilage-like substance seem to be the only cells of the cyanobiont population whose life (and also death) is aimed at protecting the population from the bactericidal action of the phenolic compounds excreted by the host plant cells. Third, the formation of different types of cells (heterocysts and VCRCWs specialized in the production of either the mucilage-like or protein-like extracellular substances) suggests the triggering of the intrapopulation regulatory signal-sensing system. There is evidence that this system is an inherent part of all developing bacterial populations. The formation of the cell forms described in this paper is probably induced by the host plant and may be considered as a mechanism of cell differentiation. The operation of a special mechanism that induces the formation of the VCRCWs specialized in the overproduction of the mucilage-like substance is supported by the obvious similarity between this phenomenon and apoptosis. The formation of such VCRCWs is likely to be a result of the long-term evolution of cyanobacterial species capable of symbiotic relations.

It can be suggested that the VCRCWs that produce the mucilaginous substance can serve as a criterion of the adequacy of the heteromorphic alterations of cells and their cooperative interactions in response to the action of environmental factors. The functional significance of the VCRCWs that are specialized in the production of the protein-like substance is difficult to estimate, as there is no evident correlation between the formation of such VCRCWs in a cyanobiont population and its development.

The ultrastructural plasticity of symbiotic cyanobacteria shows that their genetically determined capabilities are wider than it was thought earlier. These capabilities need further studies.

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