

Chapter 2

Ultrastructural Plasticity of Cyanobacteria Under Dark and High Light Intensity Conditions

Abstract This chapter deals with investigation of cyanobacteria species varying in their morphologic and metabolic properties during cultivation under different light regimes. The presented evidence suggests that the manifestations of ultrastructural plasticity in cyanobacteria grown under high light or in darkness are indicative of the build-up of certain mechanisms of adaptation to unfavorable illumination conditions: reversible thylakoid swelling; regulation of the synthesis, and degradation of glycogen; L-transformation, and heterogeneity of the responses of cells to similar stressor. The highest diversity of ultrastructural plasticity manifestations was found in species characterized by the highest versatility of metabolism. The pioneering works dedicated to the visualization of photooxidative destruction of thylakoids are included in this chapter. A special section is devoted to experiments on irradiation of the cell suspensions with visible high-intensity light.

2.1 The Structural and Functional Features of Cyanobacteria as Prokaryotic Phototrophs with Oxygenic Photosynthesis

The major property common to all cyanobacteria is their capacity for photosynthesis accompanied with the release of molecular oxygen (oxygenic photosynthesis). This process is related to the ability of cyanobacteria to use water as an electron donor for the electron transport chain to supply the cells with energy. While this property is also found in various eukaryotic organisms, such as algae and higher plants, it is untypical of other photosynthetic prokaryotes, which utilize the energy of sunlight without releasing oxygen. According to the concept formed on the basis of paleo-microbiological studies, the phylum Cyanobacteria is one of the most archaic branches of the Bacteria evolutionary tree (Paumann et al. 2005; Pinevich and Averina 2002; Pinevich 2006). However, the time of origin of cyanobacteria is

being debated. Researchers assume that it could have happened rather early, i.e., about 2.45–2.22 billion years ago (Schirmer et al. 2011). The molecular mechanisms of photosynthesis as such evolved probably during the progenote stage, and the presence of a photosynthetic apparatus is among the most important phenotypic traits of archaic bacteria (Pinevich and Averina 2002). During such a long evolutionary process of cyanobacteria, along with the development of distinctive structural and functional features of their photosynthetic apparatus, a number of mechanisms operating at the molecular genetic level have been developed to promote the adaptation to changing illumination conditions (Golden 1995; Los et al. 2008). Modern cyanobacteria, many of which are obligatory phototrophs, are well adapted to existence both in the dark (dim caves and soil) and under conditions of overexposure to light (deserts and glaciers).

The arrangement of the photosynthetic apparatus determines the specificity of the ultrastructural anatomy of cyanobacteria compared to other prokaryotes. In the scheme, designed on the basis of the ultrastructural analysis of representatives of various taxonomic groups of cyanobacteria (excluding the genera *Prochloron*, *Prochlorococcus*, and *Prochlorothrix*, as well as *Acaryochloris marina*, which differ in the pigment composition and the structural organization of the intracytoplasmic membrane structures), the major structural components of a cyanobacteria vegetative cell are shown (Fig. 2.1). The cells belong to the gram-negative morphotype: they are surrounded by a cell wall comprising a peptidoglycan layer and the outer membrane. On the surface, sheaths, sometimes capsules, paracrystalline protein or glycoprotein S-layers, pili (also termed fimbriae), and spines may be found. The intracytoplasmic membrane structures responsible for energy supply (thylakoids) fill most of the cytoplasm. These membrane structures may form a single system, termed thylakoid networks (Nevo et al. 2009). The thylakoids are lamellae formed by paired membranes containing chlorophyll *a* as a component of the photosystems. Chlorophyll *a* is the major photosynthetic pigment in cyanobacteria, except for *A. marina*, which has chlorophyll *d* as the major pigment (Miyashita et al. 1996). On the outer side of the membrane elements of the photosynthetic apparatus, phycobilisomes are located (see also scheme in Fig. 2.2). The nucleoid with (poly)ribosome associates at its periphery is located between the thylakoids, preferentially in the central part of the cell. Various structures and inclusions are present in the cells, their set depending on the species and growth conditions of cyanobacteria. The main components include gas vesicles, which promote floatation of the cell within the water column; carboxysomes (polyhedral bodies), deposits of the key enzyme catalyzing CO₂ fixation, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO); cyanophycin granules, consisting of a unique polypeptide built of the L-arginine and L-aspartate residues and acting as an alternative nitrogen source; granules of glycogen (α -granules), lipid granules (β -granules), and granules of poly- β -hydroxybutyrate acting as sources of carbon and energy; and polyphosphate granules, sources of phosphorus. There are also other inclusions, as well as various microtubules and microfilaments (Jensen 1985; Bermudes et al. 1994; Baulina and Gorelova 1996; Baulina and Gusev 1977).

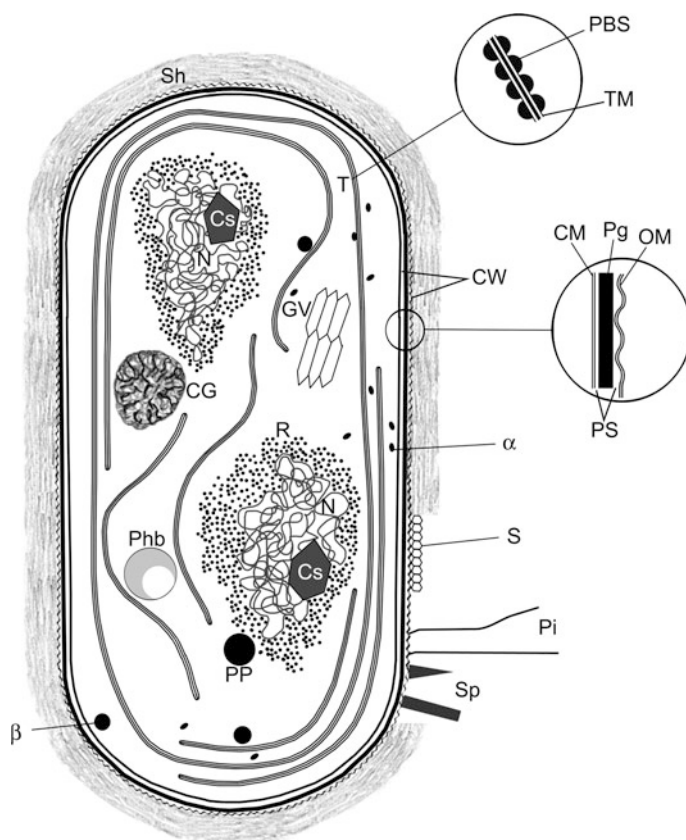


Fig. 2.1 General scheme of a cyanobacteria vegetative cell in section. α glycogen α -granules, β high electron density lipid β -granules, *CG* cyanophycin granule, *CM* cytoplasmic membrane, *Cs* carboxysome, *CW* cell wall, *GV* gas vesicles, *N* nucleoid, *OM* outer membrane, *Pg* peptidoglycan, *PBS* phycobilisome, *Phb* poly- β -hydroxybutyrate granules, *Pi* pili, *PP* polyphosphate granules, *PS* periplasmic space, *R* ribosomes, *S* S-layer, *Sh* sheath, *Sp* spines, *T* thylakoid(s), *TM* thylakoid membrane

The structural and functional organization of cyanobacteria is discussed in detail in many monographs and reviews (Drews 1973; Gromov 1976, 1986; Golecki and Drews 1982; Allen 1984; Stanier(Cohen-Bazire) 1988; Stevens and Nierzwicki-Bauer 1991; Jensen 1993; Hoiczky and Hansel 2000; Herrero and Flores 2008).

Among cyanobacteria there are obligate and facultative photoautotrophs, as well as a few species capable of switching completely to the chemoheterotrophic mode. Obligate phototrophs *Synechococcus* sp. PCC 6301 and *Anabaena variabilis* CALU 458, as well as the facultative phototrophs *A. variabilis* ATCC 29413 and *Chlorogloeopsis fritschii* ATCC 27193 are traditionally used in research and are well studied in this respect. In obligate phototrophic cyanobacteria, the ability

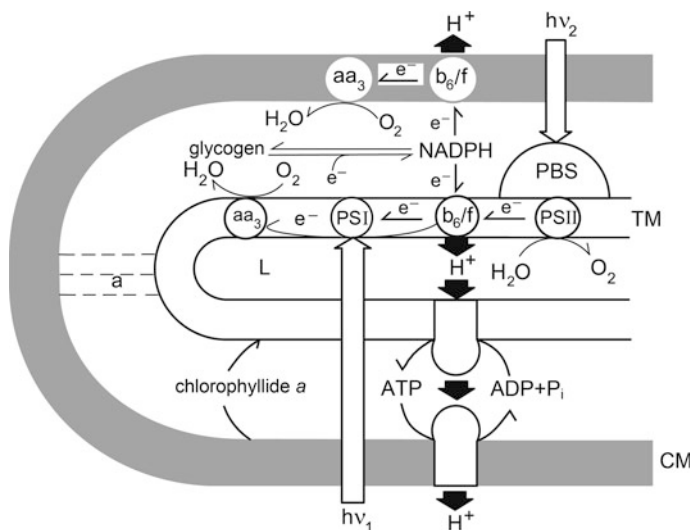


Fig. 2.2 Scheme of the major pathways of energy transfer in the membrane system of cyanobacteria [adopted from Pinevich (1997) with kind permission from author]. *CM* cytoplasmic membrane, *PBS* phycobilisome, *PSI* photosystem I, *PSII* photosystem II, P_i inorganic phosphate, *TM* thylakoid membrane, *L* lumen, *a* anastomosis between CM and TM, aa_2 cytochrome oxidase, b_6/f cytochrome b_6/f -complex. CM is dark that indicates its incompetence in light energy conversion. Outer membrane is omitted

to survive in the dark for over a month was discovered (Holm-Hansen 1968; Korzhenevskaya and Gusev 1973; Gusev and Nikitina 1974). Cyanobacteria survive the darkening periods owing to their capability to respire.

The energetic processes in cyanobacteria (photosynthesis and respiration), performed alternatively in the light and in the dark, are differentially associated with the cell membrane system including the cytoplasmic membrane (CM) and thylakoids. Numerous data on the structural and functional features of cyanobacterial membrane system are summarized in fundamental reviews and monographs (Golecki and Drews 1982; Stevens and Nierzwicki-Bauer 1991; Gantt 1994; Liberton and Pakrasi 2008; Mullineaux 2008; Nevo et al. 2009). Among them, the works of the scientific school of the St.-Petersburg State University are important, with their focusing at dynamics and adaptation of the membrane systems (Gromov 1976; Pinevich 1991, 1992, 1997; Pinevich and Averina 2002). The works using serial electron tomography combined with montaging to reconstruct large volumes of cyanobacterial cells in three dimensions show promise in the study of the whole-cell architecture developmental dynamics of the cyanobacterial membrane system (Liberton et al. 2011).

According to the modern concepts, the photosynthetic apparatus in cyanobacteria, excluding *Gloeobacter violaceus* with its photosystems incorporated in the CM, is associated with thylakoids. Paired membranes of thylakoid, appearing in ultrathin sections as two parallel elementary membranes, are either tightly packed

with their inner surfaces touching (internal adhesion) or separated, forming an intrathylakoid space (lumen). Typically, thylakoids do not touch each other, since, unlike the granal thylakoids of eukaryotic chloroplasts, thylakoids of cyanobacteria have no external adhesion factor, the chlorophyll *a/b*-binding protein of the light-harvesting complex II. Moreover, the major light-harvesting antennae of cyanobacteria are phycobilisomes, containing phycobilin pigments and transferring light excitation energy ($h\nu_2$) preferentially to photosystem II (PSII), are located at the outer surface of the thylakoids (Fig. 2.2). Phycobilisomes of most cyanobacteria are particles of semispherical shape located on the surface of the thylakoids in ordered tight rows. The spatial arrangement of thylakoids in many unicellular cyanobacteria (peripheral parallel lamellae) is more conservative than in the filamentous species and in those with complex life cycles (mostly unordered lamellae). A new model constructed using the larger-volume electron tomographic data showed a the unicellular cyanobacterium, *Cyanothece* sp. ATCC 51142 contained a band of radial thylakoids spirals around the cell periphery, forming an interconnected network derived from continuous branching and splitting of the membranes (Liberton et al. 2011). In this work, the thylakoid membranes were shown to form an extensive system enclosing a single space, the thylakoid lumen. The question of existence and organization of the structural and spatial connections between CM and the thylakoid membranes is still being debated (see Sect. 3.2). An anastomosis-like junction between these two structures was observed, albeit rarely. Specialized structures, namely, thylakoid centers, located immediately beneath the CM and connected to the thylakoids, participate in biogenesis of the intracytoplasmic membrane apparatus, particularly, in pigment transport and incorporation into the membranes (Nickelsen et al. 2011; Kunkel 1982). Functionally, cyanobacterial thylakoids are first of all energetic organelles capable of alternate photosynthetic, as well as respiratory, electron transport. On the contrary, CM contains no chlorophyll *a* and, most likely, participates only in respiration. The scheme (see Fig. 2.2) demonstrates localization of the photosynthetic apparatus and the major components of the electron transport chain in a thylakoid and in CM. Within the framework of this chapter, we should emphasize the following. First, in a thylakoid membrane, the cytochrome *b₆f*-complex positioned between the two macromolecular complexes of photosystems PSI and PSII is a common unit of the two chains performing alternate photosynthetic (light-controlled, electrons provided by PSII) and respiratory (controlled by NADPH, an electron donor of the respiratory chain) proton transport into the lumen. Second, the reducing agent may be generated not only in the course of light-controlled electron transfer, but in the process of respiratory catabolism of storage polyglucosides (glycogen). Immobilization of the storage polyglucosides occurs exclusively through the oxidative pentose phosphate pathway yielding NADPH. In the dark, the aerobic respiratory chain completes the oxidation of polyglucosides converting the stored free energy into the proton-motive force. Third, a phycobilisome is located exactly above the PSII on the outer surface of thylakoid membrane facing the cytoplasmic matrix.

2.2 The Effects of Dark and High Light Intensity Conditions on Cyanobacterial Ultrastructure

The basic data on the influence of various illumination conditions including darkness and high light intensity, on the configuration and spatial organization of the thylakoids were obtained in the early days of investigation of cyanobacterial ultrastructure. Experiments were performed using the species differing in morphology and metabolic properties, including obligate phototrophs and those capable of chemoheterotrophic growth, such as *Symploca muscorum* (Bowen and Pankratz 1963); *Nostoc muscorum* (Wildon and Mercer 1963; Ginsburg and Lazaroff 1973); *Oscillatoria chalybia* (Giesy 1964); *Oscillatoria redekei* (Whitton and Peat 1969); *C. fritschii* (Peat and Whitton 1967; Findley et al. 1970); *Anacystis nidulans* (*Synechococcus* sp. PCC 6301) (Allen 1968; Nikitina et al. 1979); *Nostoc* sp., isolated from *Macrozamia lucia* roots (Hoare et al. 1971); *A. variabilis* (Nikitina et al. 1974); *Agmenellum quadraplicatum* (Kalley et al. 1977) and some other species (Gromov 1976). In these studies, various reorganizations of the thylakoids in the cytoplasm were reported. It was found that during dying off in the dark or when exposed to high light intensity, the intrathylakoid space increased and vesicles were formed of the thylakoid membranes; moreover, in the cells turned yellowish under high light intensity vesiculation could be reversible. The present chapter is focused on a series of consecutive experiments on comparative study of the effects of darkness and high light intensity on the cell ultrastructure of the species traditionally studied by microbiologists, namely:

Synechococcus sp. Näg. PCC 6301 (hereafter referred to as *Synechococcus* sp. 6301) previously known as *Anacystis nidulans* (Rippka 1972) from the collection of the Department of Physiology of Microorganisms (Faculty of Biology, Lomonosov Moscow State University); belongs to subsection I of the phylum Cyanobacteria¹ subsection I (previously known as order Chroococcales); it is a unicellular obligate phototroph incapable of photoheterotrophic growth, diazotrophy, or cell differentiation;

Synechococcus elongatus Näg. B-267 (hereafter referred to as *S. elongatus*) is a thermophilic strain provided by the Department of Biophysics (Faculty of Biology, Lomonosov Moscow State University);

Anabaena variabilis Kütz. CALU 458 (hereafter referred to as *A. variabilis* 458) is an obligate phototrophic strain, capable of photoheterotrophic growth in the presence of glucose and some other sugars (Suleimanova 1982) but not nitrogen-fixing and forming no differentiated cells to perform this process (heterocysts); the strain was obtained from the collection of the Department of Microbiology (Biology Research Institute of St.-Petersburg State University); belongs to subsection IV (previously, order Nostocales).

¹ Bergey's Manual of Systematic Bacteriology (Boone and Castenholz 2001).

Anabaena variabilis Kütz. ATCC 29413 (hereafter referred to as *A. variabilis* 29413) [strain of Dr. C. P. Wolk, United States] is a facultative phototrophic, diazotrophic strain, capable of chemoheterotrophic growth with fructose as a source of carbon and energy (Wolk and Shaffer 1976) and of heterocyst and akinete differentiation; the strain was kindly provided by Prof. S. V. Shestakov (Department of Genetics, Faculty of Biology, Lomonosov Moscow State University).

Chlorogloeopsis fritschii Mitra et Pandey ATCC 27193 (hereafter referred to as *C. fritschii*) is a strain characterized by the most flexible metabolism if compared to the first two species; it is a facultative phototroph capable of chemoheterotrophic growth on some sugars (Fay 1965), nitrogen fixation, and cell differentiation. The life cycle comprising several stages of progressive morphology metamorphosis is characteristic of these cyanobacteria; the culture was obtained from Dr. N. G. Carr, England; it belongs to subsection V (previously, order Stigonematales).

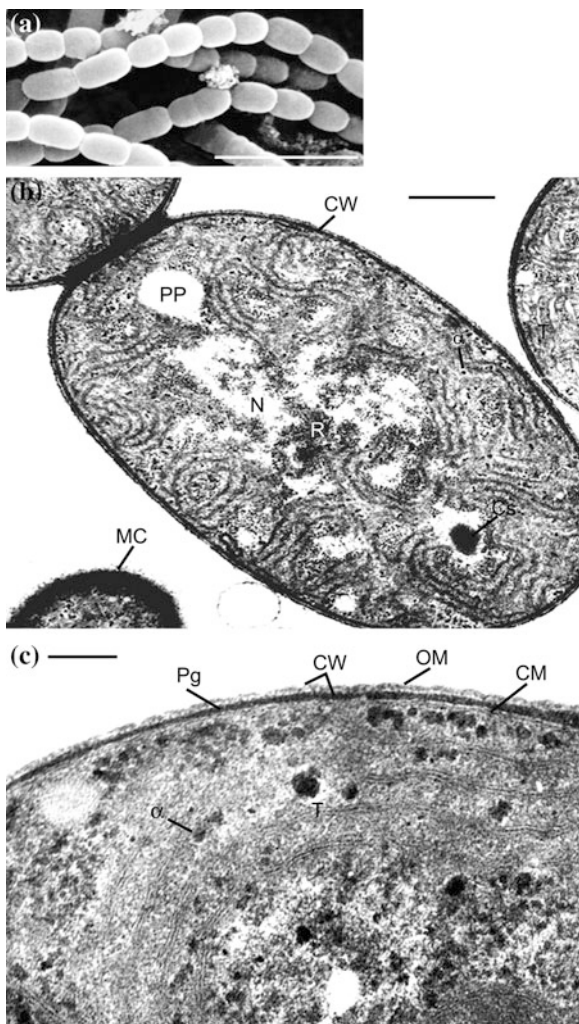
These organisms were chosen in order to study the ultrastructural plasticity of the representatives of various taxonomic groups of cyanobacteria differing in their physiological properties, which are mostly known and have been described in the literature.

2.2.1 Ultrastructure of Anabaena variabilis CALU 458 Incubated in the Dark and Transferred to the Light

For this strain, three successive stages of the culture incubated in the dark were established: (1) survival with the preservation of their capacity for proliferation upon transfer to the light; (2) preservation of the cells morphologically similar to the intact ones, yet incapable of proliferation in the light; and (3) culture lysis (Korzhenevskaya and Gusev 1976a). Study of the dynamics of the variations in the cellular ultrastructure during the first two stages revealed configurational changes in the thylakoids which could be reversed upon transfer to light after the first stage of incubation in dark (Baulina and Gusev 1978; Gusev and Baulina 1979). These ultrastructural changes may be interpreted as a result of the mechanism of reversible swelling of these organelles enabling the preservation of cell functionality in the dark, as will be confirmed below.

In these experiments, the ultrastructure of *A. variabilis* 458 during the phase of intense growth under optimal illumination conditions (1.5–2 klx) in the mineral medium was used as the control. Scanning electron microscopy demonstrated the cell morphology: ellipsoid cells joined into long chains (Fig. 2.3a). Transmission electron microscopy (TEM) revealed cell organelles, cellular structures, and various inclusions, characteristic of the species, which are depicted in the general scheme of a cyanobacterial vegetative cell (Fig. 2.1). It can be seen that *A. variabilis* 458 cells of the control culture possessed the cell wall structure

Fig. 2.3 Ultrastructure of *Anabaena variabilis* CALU 458 in the phase of intense growth in the light. General view of the cells in the culture: scanning (a) and transmission (b) electron microscopy; cell region in an ultrathin section (c). α glycogen α -granules, CM cytoplasmic membrane, Cs carboxysome, CW cell wall, MC capsular matter, N nucleoid, OM outer membrane, Pg peptidoglycan, PP electron-transparent region of polyphosphate granule localization, R ribosomes, T thylakoid. Scale bar a 10 μ m, b 0.5 μ m, c 0.1 μ m



typical of cyanobacteria, containing the outer membrane and the peptidoglycan layer (Fig. 2.3b and c). As a rule, deposits of unstable capsular material were revealed on the surface of the cells. Thylakoids filled the cytoplasm, and among them, mostly in the central region of the cell, the nucleoid with peripheral accumulations of ribosomes was located. Within the section plane, the thylakoids were located in parallel to each other and bent together. The thylakoid-forming membranes were approximately 8 nm thick each. They were either pressed together or separated from each other with a distance of some 20 nm (Fig. 2.3b). The three-layer membrane profile characteristic of the ultrastructure image was revealed (Fig. 2.3c). The cytoplasmic matrix was of high electron density which probably resulted in poor resolution of phycobilisomes on the outer surface of the

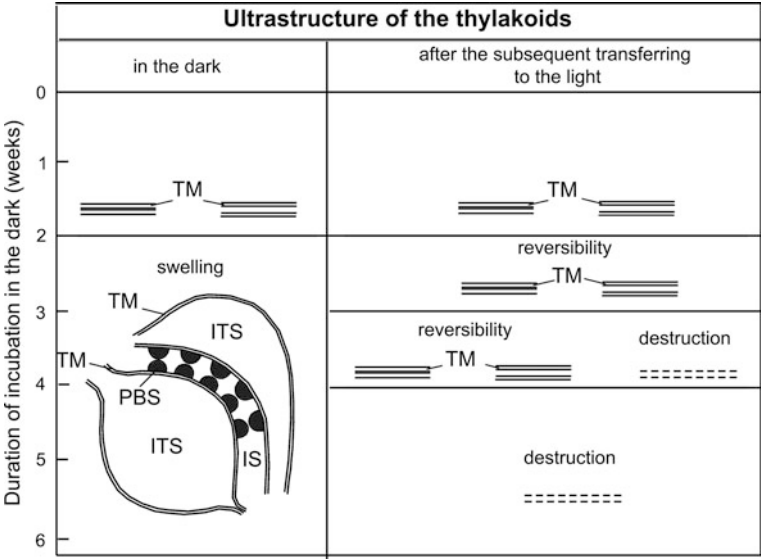


Fig. 2.4 Scheme of the ultrastructural organization of the thylakoids of *Anabaena variabilis* CALU 458 upon changes in illumination conditions. *ITS* intrathylakoid space, *IS* interthylakoid space, *PBS* phycobilisome, *TM* thylakoid membrane

thylakoids. The oval granules about 30 nm in diameter were present in the cytoplasm of all cyanobacterial species studied have been described in the literature as glycogen α -granules. The polyglucoside nature of the granules has been identified with various cytochemical techniques, as well as by biochemical analysis of the isolated granules (Fogg et al. 1973; Stanier(Cohen-Bazire) 1988). Carboxysomes, that is, deposits of the RuBisCO enzyme (Codd and Marsden 1984), were typically found in the region of the nucleoid.

After transfer of the culture to the dark and incubation for about 10 days (initial period of the first stage), the ultrastructure of the cells, including the thylakoids, did not change (Fig. 2.4). The cells retained the ability to proliferate upon return to light.

It is known from the literature data that the preservation of viability in the darkness occurs due to consumption of endogenous storage compounds, primarily glycogen, which is formed in abundance upon CO₂ photoassimilation. Catabolism of glycogen glucose occurs via the pentose phosphate cycle which is linked to the electron transport chain through NADPH (Kondrat'eva 1996) (see Fig. 2.2). The scheme of the respiratory chain was designed specifically for *A. variabilis* 458 (Pinevich 1991). In the experiments described in this chapter, at the initial stage of survival in the dark (first 2 weeks), glycogen α -granules could still be observed in the cells while the thylakoid structure was similar to that of the control cells.

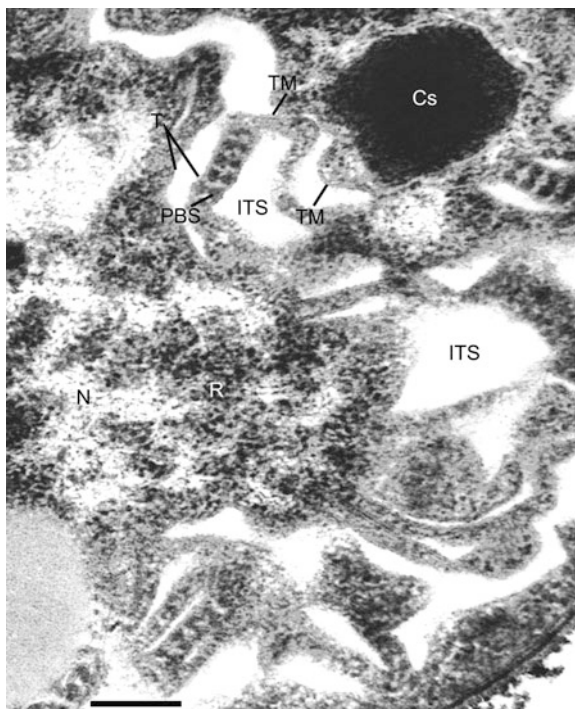
It was also demonstrated that intense consumption of storage polysaccharide occurring during this first stage of the *A. variabilis* 458 culture incubated in the dark was associated with cell survival (Korzhenevskaya and Gusev 1976a). Moreover, it was demonstrated that during the initial stages of incubation in the dark, the cells of the strain under study retained high levels of ATP synthesis (Sentzova et al. 1975). Therefore, there is firm ground for the assumption that thylakoid ultrastructure (closely connivent membranes) during the period reflects the presence of membrane-coupled energetic process of oxidative phosphorylation which occurs apparently within a chain of transformation of endogenous glycogen reserves. Similar to the energetic organelles of eukaryotic cells, this state may be termed an energized one. In the classical studies, it was demonstrated that isolated plant chloroplasts condensed upon addition of sufficient amounts of ATP into the incubation medium (Molotkovsky and Dzubenko 1969). Therefore, closely connivent thylakoid membranes and the presence of glycogen during the initial stage of incubation in the dark corresponded to a switch in the electron transport chain functioning under these conditions.

This reported interpretation of the results is in accordance with the concept of interrelation of the thylakoid configuration with formation and direction of the transmembrane proton flow in the course of cyanobacterial thylakoid electron transport chain functioning during both respiration and photosynthesis (Albertsson 1982; Pinevich and Topchieva 1991; Pinevich 1997, 2006). Membrane surfaces of the organelles are presumably brought together by adhesion via hydrophobic interactions, van der Waals bonds, and hydrogen bonds upon compensation of the negative charge with the protons released into the intrathylakoid space.

Proton entry into the thylakoid lumen of cyanobacteria is an established fact (Barsky et al. 1981; Paumann et al. 2005). The observed difference in the thylakoid ultrastructure both in a single cell and in multiple cells of the culture, in the darkness and in the light, namely, that the membranes were either tightly connivent or separated by a distance of ~ 20 nm (see Figs. 2.3b, c, and 2.4), is probably associated with the changes in adhesive properties of the membranes in accordance with the periodic functioning of the electron transport chain.

Prolonged incubation of the culture in the dark resulted in a period (within the first stage) when the cells retained their ability to proliferate upon transfer to light, although in most of them the ultrastructure changed. This was manifested mainly in the several-fold increase in the distance between the thylakoid membranes, on average, up to 100 nm or more (Figs. 2.4 and 2.5). Upon bending and possible stretching of the membranes, their typical three-layer profile was retained, indicating the absence of destructive changes and the ultrastructural integrity of the membrane lipid bilayer. Curving and stretching of the membranes may be due to the changes in the level of saturation of fatty acid residues of the membrane phospholipids determining the viscosity and fluidity of the lipid bilayer, which was observed upon transfer to the dark of a number of cyanobacterial species (Al-Hasan et al. 1989). The presence of the enzymes involved in membrane fluidity modulation (fatty acid desaturases) was demonstrated in cyanobacteria (Tasaka et al. 1996). The function of desaturases is to form double bonds between the

Fig. 2.5 Region of the *Anabaena variabilis* CALU 458 cell after incubation in the dark for 17 days (TEM). Adopted from Baulina and Gusev (1978). *Cs* carboxysome, *ITS* intrathylakoid space, *IS* interthylakoid space, *N* nucleoid, *PBS* phycobilisome, *R* ribosomes, *TM* thylakoid membrane. Scale bar 0.2 μm



carbon atoms of the fatty acid chains of the membrane lipids. It was demonstrated that in cyanobacteria illumination conditions were among the factors affecting the fatty acid composition, and therefore the fluidity of the thylakoid membrane through desaturase activity (Sciuto et al. 2008).

In our experiments, the preservation of the ultrastructural integrity of the thylakoid membranes upon stretching was also confirmed by the fact that on the membrane surface ordered phycobilisomes approximately 28 nm in diameter were visualized. These structures apparently remained connected to the membrane. In parallel experiments, it was demonstrated that phycocyanin content in the cells during this period was practically the same as in the cells of the original culture (Korzhenevskaya and Gusev 1973). At the same time, more distinct appearance of phycobilisomes if compared to the control indicated reorganization and probably even a loss of some electron-dense components of the cytoplasm in the interthylakoid space. The size of the interthylakoid space was relatively stable and corresponded to the thickness of the double layer of phycobilisomes (see Fig. 2.5).

During 3 weeks of incubation in darkness, the observed change in configuration of the thylakoids was reversible upon transfer to optimal illumination conditions in all studied cells in the population (see Fig. 2.4). After 2 days of growth in the light, which corresponds to the period of slow transformation of the culture from the lag

phase to the exponential growth phase under these conditions (Korzhenevskaya 1975), the thylakoid membranes approached each other again in accordance with their return to the energized state apparently due to resumed photosynthesis.

Taking into account the theoretical background presented in the Introduction, the ultrastructural plasticity of prokaryotic cells should be considered as a constituent of the general phenotypic plasticity, a complex of adaptive structural adjustments of the cell in response to the changes in the environment. Adaptive nature of the configurational changes in the thylakoids was indicated by the fact that they were coupled to the preservation of viability of the culture in the dark. Reversibility of the ultrastructural rearrangements of the thylakoids indicated the preservation of their structural and functional integrity. Consequently, the observed configurational changes of the thylakoids may be considered a manifestation of their ultrastructural plasticity. Apparently, reversible stretching is a form of ultrastructural plasticity of thylakoid membranes while reversible increase of the intrathylakoid space is a form of the ultrastructural plasticity of these organelles. Similar results evidencing reversibility of the degradation processes associated with iron deficiency, which include swelling of the thylakoids, were obtained for a unicellular cyanobacteria *Agmenellum quadruplicatum* in the course of investigation of their ultrastructure during cultivation (Hardie et al. 1983).

In the reported series of experiments, it was demonstrated that in those cells where a considerable increase in intrathylakoid space occurred, glycogen granules were absent (see Fig. 2.5). The correlation between thylakoid configuration and the presence of glycogen granules was observed throughout the period of incubation in the dark (up to 1.5 months). No α -granules were observed upon increase of the intrathylakoid space. In the case when the thylakoids still preserved their structure (connivent membranes), if only in sporadic cells, α -granules were typically present in the cytoplasm. The revealed pattern is in accordance with the idea that endogenous glucan is used as a source of carbon and energy during the initial period of persistence under unfavorable conditions in the dark. If the period of incubation in the dark was prolonged, the total ATP level in the *A. variabilis* 458 culture decreased significantly (Sentzova et al. 1975). Thus, these observations indicate that considerable separation of the thylakoid membranes is associated with the ceased or decreased activity of the respiratory electron transport chain and thus with the transfer of the membranes to the non-energized state. In this case, the proton flow into the lumen probably decreases (stops) and the negatively charged membranes lose their adhesive properties. A variety of interrelationships between the structural state of thylakoid membranes and proton transport has long been known for isolated chloroplasts (Packer et al. 1970; Murakami and Packer 1970). It is assumed that the changes in the configuration of the chloroplast thylakoid (swelling) are related to the movement of osmotically active ions resulting from the changes in permeability of the thylakoid membranes upon disappearance of the proton gradient required for their active transport, which may lead to water influx into the intrathylakoid space. Phosphorylation is considered the major process responsible for the changes of the chloroplast structure: swelling of the chloroplasts occurring in the dark is reversible upon transfer to light or changes in the

medium pH. Modern research confirmed and significantly augmented our understanding of the nature and mechanism of thylakoid swelling in the chloroplasts of higher plants in the dark (Johnson et al. 2011).

Comparison of the literature data on the mechanisms of light-dependent changes in the structure of chloroplasts and the facts on the behavior of the obligate phototroph *A. variabilis* 458 in the dark listed above suggest that the hypertrophy of the intrathylakoid space in this cyanobacterium reflects the process of thylakoid swelling in the dark due to slowing down of electron transport and the associated proton transport. The swelling process is apparently associated with the ability of the membranes to stretch. Indirectly, this is supported by the fact that in the cells of a chemoheterotrophic culture *C. fritschii* grown in the dark where the electron transport chain most probably functions, no thylakoid swelling was observed (Baulina et al. 1978). Therefore, the reversible configurational changes in the thylakoids of *A. variabilis* 458 revealed upon its long-term incubation in the dark, may be explained by the osmotic mechanism linked to the light-dependent changes in the intensity of electron transport. This hypothesis is in agreement with the interpretation of the morphological and functional dynamics of the membrane apparatus of cyanobacteria (Pinevich 1991, 1997), while the results obtained in the model system described above indicated, as was expected, that the ultrastructure plasticity of thylakoids and constituent membranes was associated with the metabolic rearrangements under varied illumination conditions.

The leading role in various types of structural and functional reorganizations in the thylakoid system of cyanobacteria is often assigned to the redox status of the electron transport chain, which transmits external signals, such as changes in the intensity and quality of light, to various epigenetic-level regulatory systems (Pinevich 1991). Moreover, evidence exists suggesting that illumination conditions regulate expression of the genes coding for the components of PS II complex and phycobilisomes via the photoreceptors specific for the light of different wave lengths and involving the electron transport chain and the photosynthesis apparatus as such (Golden 1995). The state of cyanobacterial photoreceptors, including at least one putative rhodopsin-like chromoprotein (Hoff et al. 1995), may be in connection with the activity of specific transcription regulators (Bazanov and Pinevich 2000). In purple photosynthesizing bacteria *Rhodobacter*, a protein sensitive to blue light and redox-dependent signal and involved in the initiation of regulatory processes, inducing gene expression was detected (Braatsch et al. 2002, 2004). In recent years, the process of light-dependent regulation of the physiological state of cyanobacteria has been studied intensely (Montgomery 2007).

The recent data support a conclusion that redox signals formed in a bacterial cell, that is, the signals evolving upon the changes in the redox status, are received by various regulatory systems able to control numerous metabolic functions including photosynthesis, carbon and nitrogen fixation, as well as aerobic and anaerobic respiration, to maintain the homeostasis and adaptation of cell (Kaplan 2004). Cyanobacteria possess also the regulatory systems triggered by specific sensors, e.g., the membrane proteins reacting to changes in such parameters of the membrane physical state as fluidity, independently of the nature of the stress signal

(Los and Murata 2004). Basing on these general concepts, light-dependent reversible changes in the configuration of thylakoids may be considered as a process involving the regulatory systems at various levels, including osmoregulation and expression or modification of the enzymes involved in reorganization of the membrane phospholipids. Thus, the manifestation of ultrastructural plasticity of the thylakoids (reversible divergence of their membranes and considerable increase in the intrathylakoid space) in the obligate phototroph *A. variabilis* 458 at the stage of survival in the dark indicates a complex adaptive mechanism of reversible swelling of these organelles. Deciphering of the molecular genetic basics of this mechanism is far from complete.

As is known from the literature, the absence of light, if only for a short time, induces reorganization of the pigment-protein complexes of PSII in obligate phototrophic cyanobacteria (Lebedev et al. 1989). Duration of the swelling adaptive mechanism functioning during the period of the preservation of cell viability in the dark probably correlates with the intensity of destructive changes in the photosynthesis apparatus, primarily in PSII, or, in other words, with the ability to repair the darkness-induced damage and turn on the photosynthetic electron transport chain upon transfer back to light. It was demonstrated for *Synechocystis* sp. PCC 6803 that cyanobacterial cells were able to repair PSII defects (caused by ultraviolet light) by means of a reparation mechanism based on de novo synthesis of D1 and D2 polypeptides (Friso et al. 1994). However, it should be taken into account that molecular mechanisms of the adaptation of the photosynthetic apparatus to stress in cyanobacteria and plants are multifactor and complex [see review (Kreslavski et al. 2007)].

Further studies of the behavior of the model described in this chapter during the period designated as the second stage demonstrated that adaptive abilities of obligatory phototrophic cyanobacteria may be limited by the damaging effects of light on the cells weakened by incubation in the dark. Starting from week 4, transfer of the cyanobacterial culture back to the illumination conditions optimal for growth resulted in a loss of viability of most of the cells and development of the degradation processes (Korzhenevskaya 1975). After five and more days of incubation under the light of moderate intensity, bleaching of the culture was observed. Deep destructive changes in the thylakoids were observed by TEM: the membranes lost their three-layer profile and appeared as vague outlines (see Figs. 2.4 and 2.6) (Baulina et al. 1976, 1977; Gusev and Baulina 1979). In these cells, both intra- and interthylakoid space was filled with homogenous fine-grained cytoplasm content, while the phycobilisomes were destroyed. All the cytoplasm was apparently in a homogenized state, no α -granules or ribosomes could be revealed. The nucleoid was detected only in rare sections in the central part of the cell (Fig. 2.6a). As a rule, carboxysomes were preserved in the nucleoid zone. Biochemical analysis revealed considerable destruction of DNA, RNA, proteins, and pigments (Korzhenevskaya and Gusev 1976b). On the basis of these data, the authors of this work proposed that the reason for destructive changes in the cellular components accompanied by bleaching of the culture was photooxidation induced by light of moderate intensity after long-term incubation in the dark. The outer

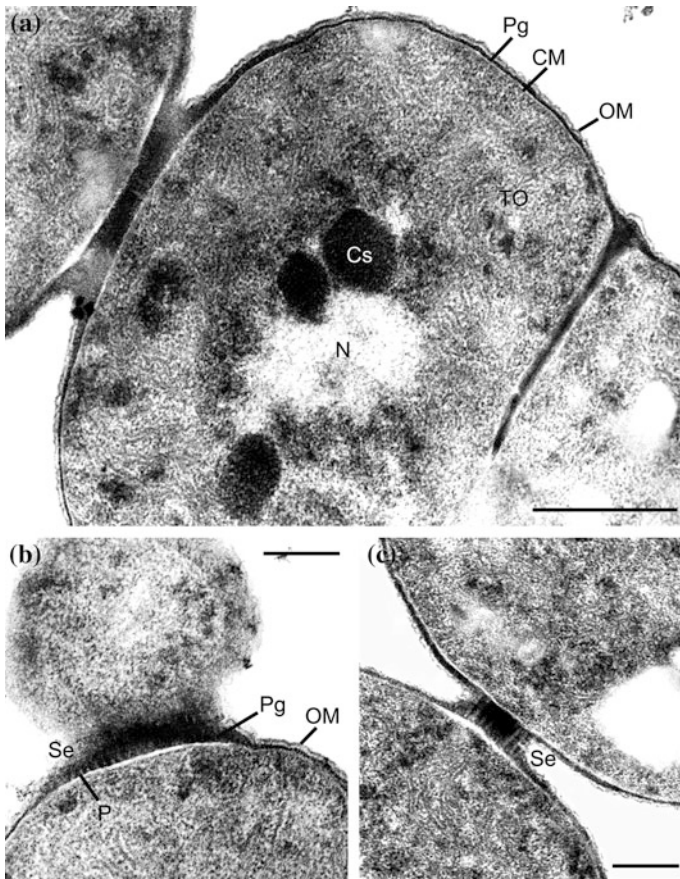


Fig. 2.6 Ultrastructure of *Anabaena variabilis* CALU 458 cells in the culture after 1.5 months of incubation in the dark followed by 5 days of cultivation in the light: general view (a) and regions adjacent to the septum (b, c) (Baulina et al. 1977). *CM* cytoplasmic membrane, *Cs* carboxysome, *N* nucleoid, *OM* outer membrane, *P* pore-like structures in the cell wall peptidoglycan layer, *Pg* peptidoglycan, *Se* septum, *TO* outlines of the thylakoids. Scale bar **a** 0.5 μm , **b** 0.2 μm , **c** 0.2 μm

membrane of the cell wall and the CM turned out to be the relatively stable membrane structures. At the same time, in most cells reorganization of the structure of the septum-forming peptidoglycan layer were evident. Numerous evenly alternating electron-transparent pore-like regions were revealed. Depending on the direction of the ultrathin section, these structures may be revealed at the periphery of the septum (Fig. 2.6b); in this case they resemble the junctional pores involved in mucus secretion in gliding cyanobacteria including another *A. variabilis* strain (see Sect. 5.1.2). In the sections of the central part of the septum, pore-like formations were revealed distinctly as canaliculi in the thick peptidoglycan layer (Fig. 2.6c). It should be noted that occasionally the intercellular channels were observed in the control *A. variabilis* 458 culture grown under optimal

conditions. No connection between these structures and CM was detected; they therefore cannot be identified as microplasmodesmata, which are considered to be present in the septa between the vegetative cyanobacterial cells, as well as in the region of their contact with heterocysts (Lang and Fay 1971; Merino et al. 1994; Flores et al. 2006). Electron-transparent channels similar to microplasmodesmata were revealed in the septum peptidoglycan between the vegetative cells, as well as between the vegetative cells and heterocysts in trichomes of a symbiotic cyanobacterium *Nostoc* sp. f. *Blasia* upon long-term storage in the dark at low temperature (Gorelova and Baulina 2009). Ultrastructural plasticity of this cyanobacterium is described in Chap. 5. Study of a symbiotic cyanobacterium in *Azolla caroliniana* ferns also revealed the structures identified as microplasmodesmata between the vegetative cells and heterocysts (Braun-Howland and Nierzwicki-Bauer 1990). Internal organization of the channels similar to microplasmodesmata remains poorly studied, although occasionally strands of electron-dense matter contacting with CM were revealed in them (Flores et al. 2006; Gorelova and Baulina 2009). The role of the latter in channel formation is, however, not strictly proved.

An ultrastructural manifestation of destruction of the thylakoids and other cytoplasm components similar to the one described above was revealed in an *Anacystis nidulans* L 1402-1 (SAG) (*Synechococcus* sp.) culture in the light in the presence of O₂ and under some additional conditions enhancing the photooxidation processes (Peschek and Schmetterer 1978; Schmetterer et al. 1983).

Transfer of a 3–4-week-old *A. variabilis* 458 culture from the darkness to the light exhibited cell heterogeneity in the population since only some of the cells underwent destruction in the light, while reversible changes occurred in the remaining ones. At the same time, the studied samples of the culture transferred to the light after incubation in the dark for 1.5 months contained only the cells described above, in which photooxidative destruction apparently occurred. In the described system, such cells lost their ability to proliferate but did not undergo autolysis during a long period of time (up to 4 months of incubation in the light); in other words, they were preserved in this state. The absence of lysis in most *Anacystis nidulans* (*Synechococcus* sp.) cells during photooxidation in the case of total experiment duration up to 5 days was also observed in the work cited above (Schmetterer et al. 1983). In our opinion, rather interesting and important is the ultrastructural similarity between the cyanobacterial cells with the described type of destruction and the mummified cells of other microorganisms, in particular *Micrococcus luteus*, which were formed under the influence of a chemical analog of an anabiosis autoinducer (Suzina et al. 2001). In both cases, the cells retained their shape changes in the cell wall structure occurred, which, however, did not lead to its destruction; on the contrary, cell membranes were totally destroyed, DNA and RNA degraded, and the cytoplasm became homogenous and fine grained. All these destruction processes were not accompanied by autolysis. It is assumed that the absence of autolysis in mummified cells results from enzyme inactivation with anabiosis-inducing agents, alkylhydroxybenzenes, or chaotropic salts known to damage the three-dimensional structure of the macromolecules, i.e., proteins, DNA, and RNA, and denature them, also

Fig. 2.7 Ultrastructure of an *Anabaena variabilis* CALU 458 cell in the first transfer of the culture from a flask where secondary growth was detected (see text for explanation). *ITS* intrathylakoid space, *N* nucleoid, *R* ribosomes, *T* thylakoid. Scale bar 0.5 μm

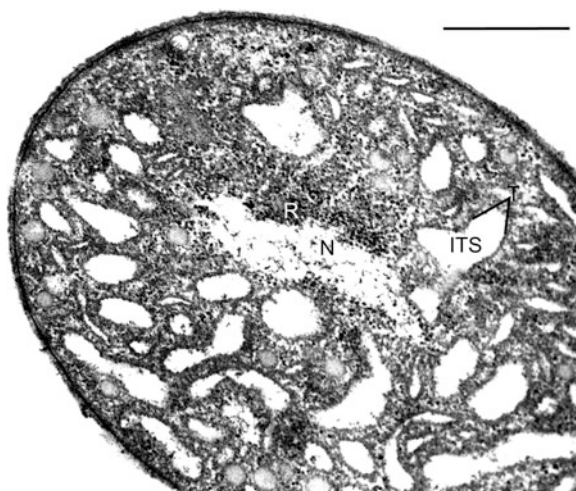
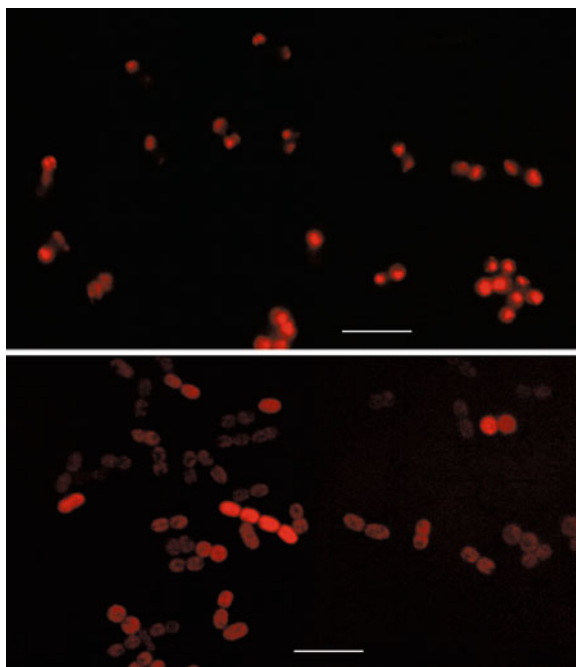


Fig. 2.8 The fluorescence of chlorophyll in *Anabaena variabilis* CALU 458 cells under a fluorescence microscope upon irradiation with ultraviolet light. In the first transfer of the culture from a flask where secondary growth was detected (see text for explanation) (a) and in the culture grown under optimal light intensity (b). Scale bar a, b 10 μm



causing similar changes in microorganisms (Duda et al. 2004). Similarly to this, conservation of *A. variabilis* 458 in the experiments described above may be due to inactivation of hydrolytic enzymes accompanied by degradation of other macromolecules caused by photooxidative damage.

Upon complete bleaching of the *A. variabilis* 458 culture, after a period of 6–42 days, in some experimental flasks, singular, very small blue–green colonies

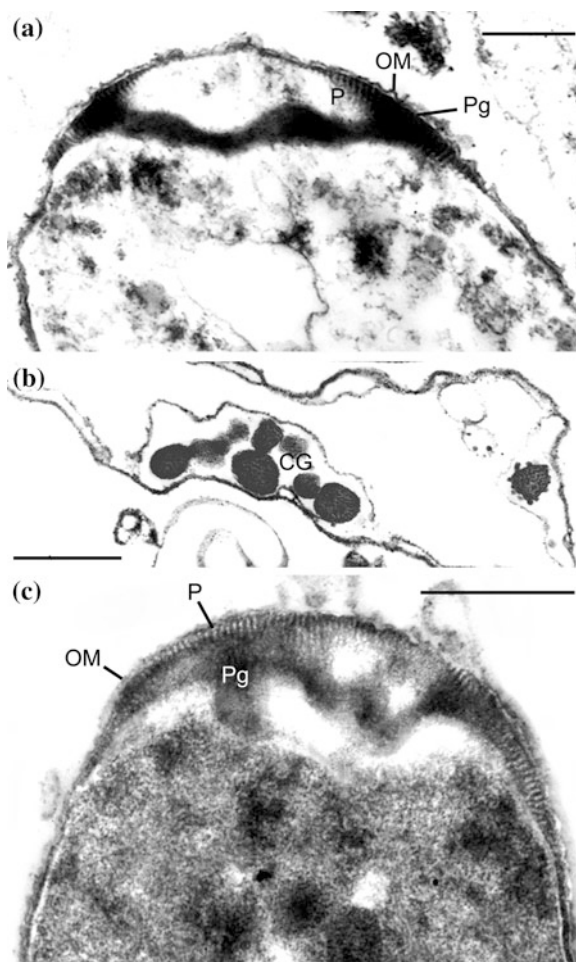
were observed, consisting of viable cells initiating the secondary population growth. It is known that the individuals in bacterial populations differ by the resistance to external stimuli. Prior to complete death of a culture, viable cells are present, which may revive the population under favorable conditions (Rabotnova 1980). In experiments with *A. variabilis* 458 in the flasks where secondary growth was observed, cells with swollen thylakoids were found after 2–4 weeks and upon the first transfer of the culture. In serial sections of these cells, a distinct tendency to the closure of the thylakoid membranes was revealed indicating their ability to form vesicles (vacuoles) (Fig. 2.7). These changes could not be revealed after the following transfers, which probably indicates their reversible nature. The phenomenon of swelling and vesiculation of the thylakoids correlated with the changes in the fluorescence of chlorophyll under a fluorescence microscope upon irradiation with ultraviolet light (Fig. 2.8a) if compared to the control (Fig. 2.8b). In the control cells, the red emission of chlorophyll was of moderate intensity and rather evenly spread, while in the experimental samples brighter local fluorescence was observed.

Thus, the reported results of ultrastructural research made it possible to visualize the consequences of oxidative destructive processes. Their initial stages are probably linked to irreversible degradation of the photosynthetic apparatus upon cell long-term growth in the dark accompanied by damage (bleaching) of the pigments after transfer to light of moderate intensity. To confirm that the interpretation of the described ultrastructural picture of thylakoid destruction as a result of photooxidative damage of the photosynthetic apparatus, it seemed reasonable to study the same cyanobacterial strain upon growth under photooxidative conditions, that is, under light of extremely high intensity in the presence of air oxygen.

2.2.2 Ultrastructure of *Anabaena variabilis* CALU 458, *Synechococcus* sp. PCC 6301 and *Chlorogloeopsis fritschii* ATCC 27193 Grown Under High Light Intensity

Cyanobacteria, similar to eukaryotes, developed various systems of protection against oxygen toxicity caused by formation of its reactive species, obviously expected under high flows of sunlight (Abeliovich et al. 1974; Merzlyak 1989; Obinger et al. 1998). However, despite the efficient mechanisms of photoadaptation, in many situations cyanobacteria are subjected to damages which are often irreversible (Abeliovich and Shilo 1972; Abeliovich et al. 1974; Eloff et al. 1976; Korzhenevskaya and Gusev 1976b; Sinha et al. 2002) this phenomenon was termed photooxidative death (Abeliovich and Shilo 1972). Molecular mechanisms of photodamage to the photosynthesis apparatus and the membranes are relatively well studied in eukaryotic cells (Merzlyak 1989), and to a lesser extent, in prokaryotes (Pinevich and Averina 2002). On the basis of these works, visualization of the destructive changes of the photosynthetic membranes in cyanobacteria

Fig. 2.9 Ultrastructure of *Anabaena variabilis* CALU 458 at late stages of destruction in a 12-day culture incubated under high light intensity (**a, b**) and upon transfer to light of optimal intensity after preliminary growth in the dark for 1.5 months (**c**): cell regions close to the septa with pore-like structures in the peptidoglycan layer (**a, c**) and cyanophycin granules remaining after cell autolysis (**b**). [**a, b** adopted from Baulina et al. (1981a)]. *CG* cyanophycin granule, *OM* outer membrane, *P* pore-like structures in the cell wall peptidoglycan layer, *Pg* peptidoglycan. Scale bar **a, b, c** 0.5 μm



under conditions of photooxidative stress is of particular importance for further detection of this process not under the controlled experimental conditions but in situ as well. Investigation of cyanobacteria of different taxonomic position, metabolic abilities, and, most importantly, level of dependence on light energy, is necessary to identify the specifics of manifestation of the photooxidative destruction, as well as of the cellular protective mechanisms. That is why in addition to the filamentous *A. variabilis* 458 capable of photoheterotrophic growth, unicellular obligate photoautotrophic cyanobacterium *Synechococcus* sp. 6301 and *C. fritschii*, which is capable of photoheterotrophic growth and possesses a complex life cycle, were also used in the comparative study of the effects of high-intensity light. The cells grown under light intensities of 9–10 klx in a mineral medium (Baulina et al. 1981b) or in the presence of glucose and ribose as additional carbon sources (Baulina et al. 1982) were studied by electron microscopy.

High light intensity suppressed considerably the growth of *A. variabilis* 458 in mineral medium (Suleimanova and Mineeva 1981). Electron microscopy showed that after 12 days of cultivation most cells underwent autolysis. To elucidate the primary changes in the ultrastructure, cell samples collected at days 2, 4, 7, and 12 of cultivation under high light intensity were studied. On day 2, in most cells thylakoid vesiculation was observed and lipid β -granules appeared (the latter are shown on the scheme in Fig. 2.1). The deposition of lipid granules could be caused by destruction of the thylakoid membranes (de Vasconcelos and Fay 1974). Cells at various stages of considerable destructive changes were observed rarely in 2- and 4-day cultures, while they predominated in the cell population of older cultures. At the ultrastructural level, these changes were observed as degradation of the thylakoid membranes and CM, formation of the characteristic pore-like structures described above in the peptidoglycan layer of the septa, as well as further destruction of the cytoplasm content, including the nucleoid, and cytoplasm autolysis with the relative intact cell wall (Fig. 2.9a). Cyanophycin granules, a form of deposition of a nitrogen-containing storage polypeptide multi-L-arginyl-poly-L-aspartic acid (Simon 1971), turned out to be the most stable structures (Fig. 2.9b). Parallel study of the effect of high light intensity on the state of the pigments demonstrated that under the experimental conditions chlorophyll and phycocyanin degraded rapidly (more than a threefold decrease in content) while carotenoid content decreased much less (1.8 times) (Suleimanova 1982).

As was demonstrated in the two models described above, the ultrastructural manifestations of the *A. variabilis* 458 cell destruction induced by moderate light after long-term incubation in the dark or by high-intensity light had both similar and different features. The picture of thylakoid membrane destruction accompanied by the loss of cell viability was similar in both cases. The major differences were the absence of autolysis in the cells subjected to photodestruction upon transfer from darkness to the optimal illumination conditions (Fig. 2.9c) during the whole period of observations and, on the contrary, deep autolysis of damaged cells upon cultivation under high light intensity (see Fig. 2.9a).

When grown in mineral medium under high light intensity, *Synechococcus* differed from *A. variabilis* 458 in the character of the ultrastructural changes. General view of the cells of a unicellular rod-shaped *Synechococcus* sp. 6301 grown under optimal illumination conditions of 1.5–2 klx (control) is presented in Fig. 2.10a. On day 20 of cultivation under 9–10 klx (extreme illumination conditions for the culture), a decrease in the growth rate was distinct (Suleimanova and Mineeva 1981), although no signs of cell autolysis were present. The culture contained two types of cells different in the ultrastructure of their cytoplasm. Cells of the first type were prevailing and analogous to those grown under optimal illumination conditions. The ultrastructure of the latter was considered as the control (Fig. 2.10b). In ultrathin sections of the cells of the first type, as well as of the control ones, the thylakoids (three to five) appeared as concentric circles organized in parallel with each other and with CM at the periphery of the cell. The thylakoid membranes touched each other tightly; the ultrastructure of each one of them (two electron-dense layers and one electron-transparent one between them)

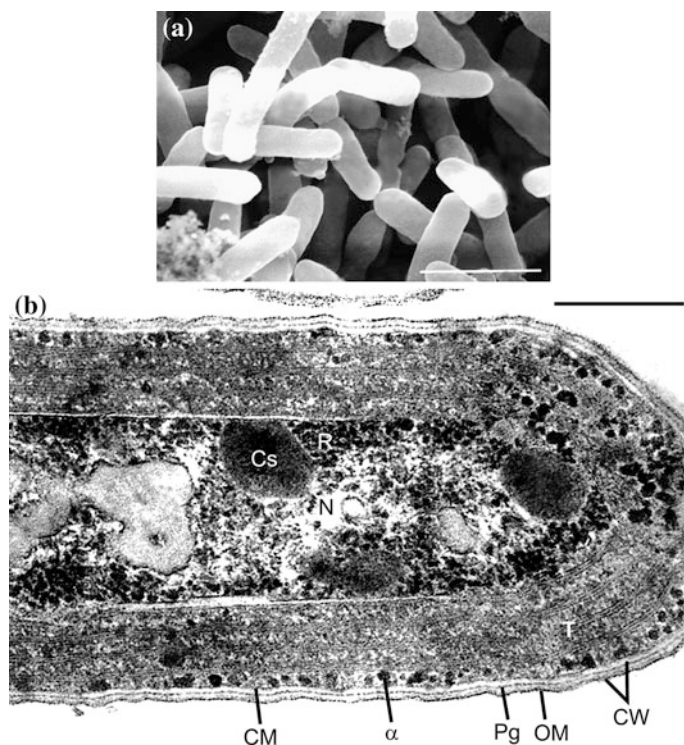
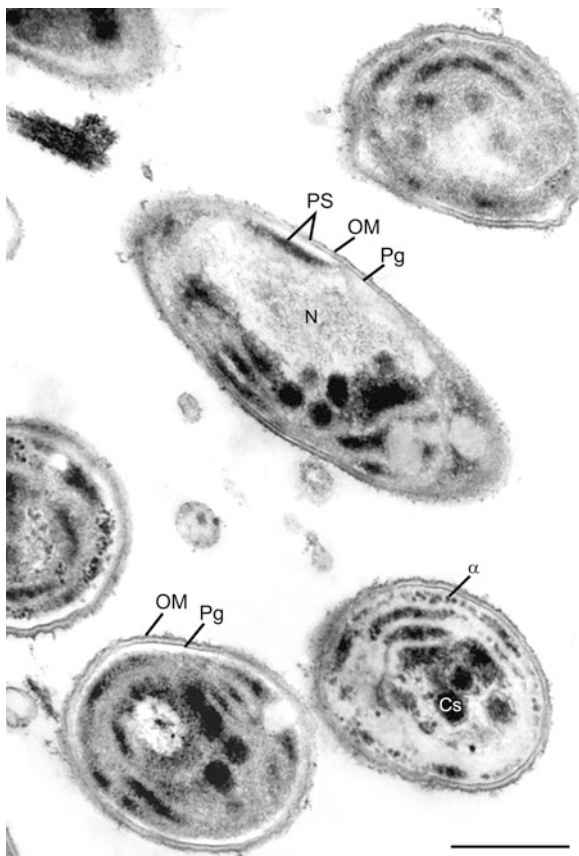


Fig. 2.10 Ultrastructure of *Synechococcus* sp. PCC 6301 grown under optimal illumination conditions: general view of the cells in the culture, SEM (a) (Baulina et al. 1994) and cell region in an ultrathin section (b) (Baulina et al. 1981a). α glycogen α -granules, CM cytoplasmic membrane, Cs carboxysome, CW cell wall, N nucleoid, OM outer membrane, Pg peptidoglycan, R ribosomes, T thylakoid. Scale bar a 3 μ m, b 0.2 μ m

corresponded to the molecular organization of a typical elementary membrane. No increase in the intrathylakoid space was observed. The cytoplasm matrix of the interthylakoid space was granular, containing α -granules of glycogen, although phycobilisomes could not be revealed distinctly on the outer surface of the thylakoids. Ribosomes filled all the space between the innermost thylakoid and the thin-fibrillar nucleoid. Carboxysomes were localized in the region of the nucleoid in the central part of the cell. Distinct separation between the central and peripheral parts of the *Synechococcus* sp. 6301 cells was typical for the species, whose ultrastructure has been studied by many researchers at the early stage of electron microscopic investigation of cyanobacteria [(Allen 1968; Fedorov and Tomina 1969) etc.].

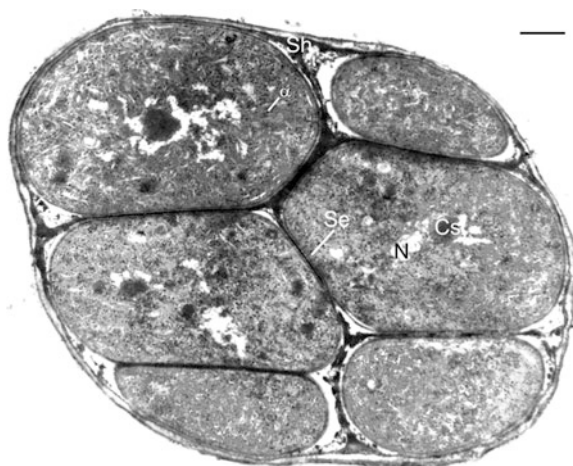
Certain cells in the culture (cells of the second type) differed dramatically from the control ones (Fig. 2.11). The thylakoid membranes were destroyed and practically indistinguishable from the homogenous fine-grain cytoplasm. Glycogen granules were not revealed in most of these cells, no ribosomes accumulated at the

Fig. 2.11 Degrading *Synechococcus* sp. PCC 6301 cell in a 20-day culture incubated under high light intensity (TEM, data obtained by Baulina, Suleimanova and Mineeva). α glycogen α -granules, *Cs* carboxysome, *N* nucleoid, *OM* outer membrane, *Pg* peptidoglycan, *PS* periplasmic space. Scale bar 0.5 μ m



periphery of nucleoid were observed. Demarcation of the nucleoid and the peripheral cytoplasmic zones was disrupted; fusion of the nucleoid zone and the periplasmic space was observed in some cells. CM was not revealed. At the same time, the nucleoid retained its fibrillar structure and, as is typical of cyanobacteria, was associated with carboxysomes whose granal surfaces were somewhat flattened and the surrounding proteinaceous outer monolayer shell was not revealed. The ultrastructure of the cell wall did not change. No cells with electron-transparent lysed cytoplasm were observed during the experimental period of growth under high light intensity. Parallel spectrophotometry of the culture revealed a decrease in the levels of chlorophyll and phycocyanin by 1.7 and 1.9 times, respectively, while carotenoid content during the phase of growth retardation practically did not change (Suleimanova and Mineeva 1981). These data, together with the induction curves, decay kinetics, and light curves recorded for delayed luminescence, characterizing the functional state of the photosynthetic apparatus (Suleimanova and Markarova 1980), definitely indicated the changes in its functioning in *A. variabilis* 458, *Synechococcus* sp. 6301 and *C. fritschii*. According to other

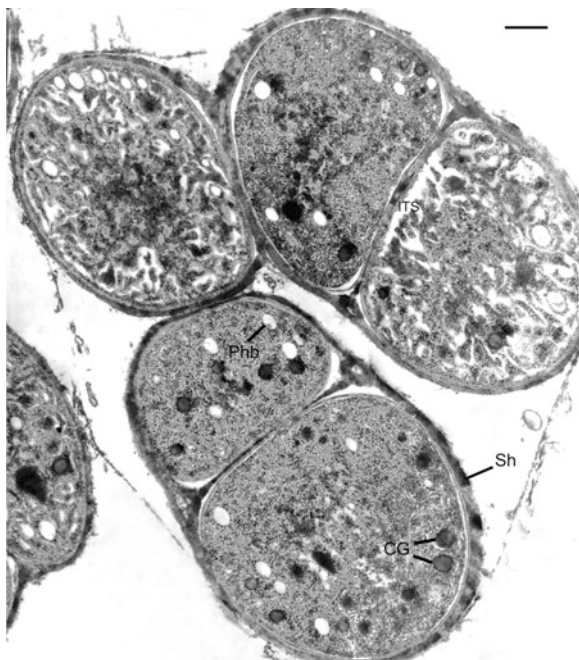
Fig. 2.12 Dividing *Chlprgloeopsis fritschii* ATTC 27193 cells joined by a common sheath at the stage of intense culture growth under optimal illumination conditions (TEM). α glycogen α -granules, Cs carboxysome, N nucleoid, Se septum, Sh sheath. Scale bar 0.5 μ m



authors, *A. variabilis* B 337 cultivation under conditions of intense illumination resulted in pigment bleaching accompanied by a significant decrease in the amount of the intramembrane macromolecular complexes corresponding to PS II and inhibition of the photosynthetic activity (Sidirelli-Wolf et al. 1992). Upon modeling of photooxidative conditions in *Anacystis nidulans* L 1402-1 (SAG) (*Synechococcus* sp.) with various metabolic inhibitors, CO₂ limitation, and elevated temperature, the degradation of the thylakoid membrane was coupled to the loss of PS II activity and chlorophyll bleaching (Schmetterer et al. 1983).

In contrast to *A. variabilis* 458 and *Synechococcus* sp. 6301, in *C. fritschii* growth inhibition under high light intensity in the mineral medium was insignificant and occurred only by the end of the phase of intense growth (Suleimanova and Mineeva 1981). No destruction of thylakoids, CM, or other structural components of the cell similar to the one described above was observed during this period (20 days). However, if compared to the control culture grown under moderate light (Fig. 2.12), the cells with swollen and vesiculated thylakoids dominated (Fig. 2.13). It should be noted, however, that *C. fritschii* cells grown under various illumination conditions could differ considerably in their thylakoid ultrastructure even in the case of the cells of similar size surrounded by a common sheath. Thylakoid membranes in some cells may be pressed to each other, while in others they were separated by a more or less wide intrathylakoid space. Moreover, the configuration of thylakoids on ultrathin sections indicated possible closure of the membranes of these organelles with formation of vesicles, or vacuoles. Such picture is presented in Fig. 2.7. These changes in the thylakoids happen in some *C. fritschii* cells under both moderate and intense illumination. It should be emphasized that thylakoid swelling and vesiculation probably reflect a nonspecific reaction of cyanobacteria not only toward unfavorable conditions of illumination but also to nutrient shortage (e.g. lack of nitrogen or iron), or may result from the degradation changes in the cells upon long-term incubation under stationary

Fig. 2.13 Ultrastructure of the cells of a photoautotrophic *Chlorogloeopsis fritschii* ATCC 27193 culture grown under high light intensity (Baulina et al. 1981a). *CG* cyanophycin granule, *ITS* intrathylakoid space, *Phb* poly- β -hydroxybutyrate granules, *Sh* sheath. Scale bar 0.5 μ m



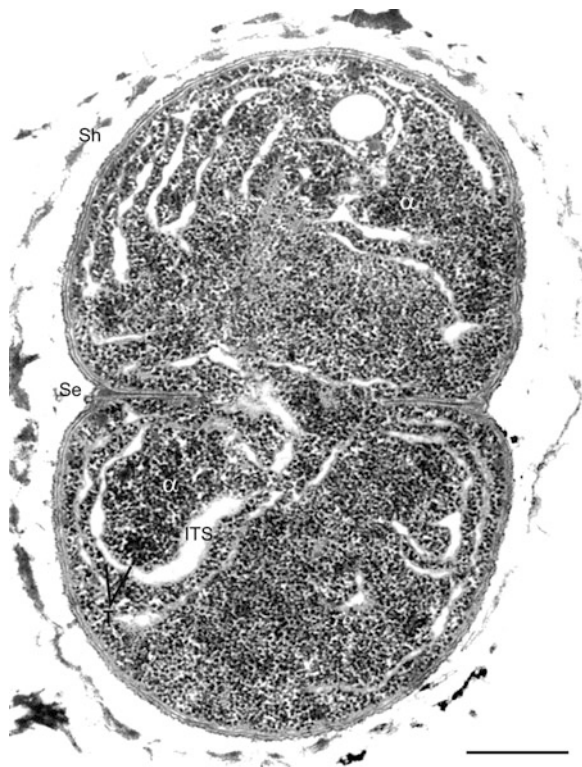
conditions (Nikitina et al. 1974); (Ermakova et al. 1977; Hardie et al. 1983; Balkwill et al. 1984; Stevens and Nierzwicki-Bauer 1991). Some researchers reported reparation of the indicated changes in the thylakoid structure upon transfers (Hardie et al. 1983) or introduction of the deficient nutrient to the growth medium (Balkwill et al. 1984). At the same time, apparently, swelling and vesiculation is a non-obligatory stage of destructive changes in the thylakoids (Stevens et al. 1981).

Thus, comparative study of the ultrastructure of three cyanobacterial species during the initial period of the inhibition of culture growth by high-intensity light demonstrated that they differed significantly in their response to the same stimuli. High light intensity had the smallest effect on the ultrastructure of *C. fritschii*. No damage visible as destruction of the thylakoid membrane, CM, and other cellular components, which could be attributed to photooxidative reactions, was detected at the ultrastructural level. Decrease in chlorophyll and phycocyanin content was practically the same as in *Synechococcus* sp. 6301, carotenoid content did not change (Suleimanova 1982). The presence of the cells with significant vesiculation of the thylakoids in the culture was probably associated with the initial stages of the functional impairment of the photosynthetic apparatus. The important peculiarity of *C. fritschii* was the presence of cells with various thylakoid structure (compressed, swollen, or vesiculated) independently of the culture age and illumination condition. These data are in accordance with the results of the studies on the effect of illumination intensity on the ultrastructure of *C. fritschii* grown at high

temperature (45 °C) (Findley et al. 1970). In the cited work, it was also demonstrated that under high light intensity thylakoids swelled, lost their parallel orientation, and formed vesicles. The authors suggested that there should be a correlation between the organization of the thylakoid system, chlorophyll content, and photosynthesis rate, as well as between the ultrastructure and chemical composition of the thylakoids under various illumination conditions. The experiments described in the current chapter demonstrated that, on the contrary, in *Synechococcus* sp. 6301, thylakoid membranes were either connivent (under illumination intensity of 2 and 9 klx) or destroyed (9 klx). Swelling and vesiculation were not observed. In general, the *Synechococcus* sp. 6301 population was relatively stable. Most cells did not change ultrastructurally; the number of concentric thylakoids decreased in some cells (from 4–5 to 3). A similar observation, that is, a decrease in the number of thylakoids and lowered chlorophyll content under increased light intensity, was made in a classical study of the ultrastructure of the same species (strain) (Allen 1968). At the same time, in the described experiments deep destructive changes could be revealed in the thylakoids, CM, and other components of the cytoplasm of some cells, although they were not associated with rapid autolysis of the cytoplasmic content, at least during the period of observation. No intermediate forms with changes in spatial arrangement, swelling, or vesiculation of the thylakoids was observed. In *A. variabilis* 458, similar deep destruction of thylakoids, CM, and other intracellular components occurred. However, in contrast to *Synechococcus* sp. 6301, it was most likely preceded by thylakoid vesiculation, which occurred already on days 2–4 of cultivation, and was accompanied by the changes in the peptidoglycan layer ultrastructure characteristic of *A. variabilis* 458. Moreover, destruction of most of the cells occurred earlier and was coupled to cell autolysis revealed during the earlier period of observations.

The phenomenon of photooxidative death of cyanobacteria had been known for a long time (Abeliovich and Shilo 1972). PS II is the primary target under illumination stress. The occurring sharp suppression of photosynthesis, or photoinhibition, is a combined process of photoinactivation of non-cyclic transport of electrons and degradation of the D1 subunit of the PS II macromolecular complex (Pinevich and Averina 2002). Photoinhibition is determined by the length of exposure to light and includes a reversible stage when repairation is possible, which is followed by the irreversible phase of photodamage. Subsequently, accumulation of degradative oxidative processes may occur, such as photooxidative destruction, caused by formation of excessive reactive oxygen species and the associated processes of lipid peroxidation, resulting in significant changes in the characteristics of the membranes (Merzlyak 1989). It is assumed that the capacity of the pigment–protein complexes for repairation provides for the reversible nature of the damage. D1 is the most actively renewed membrane polypeptide under normal illumination conditions. Expression of the *psbA* gene coding for D1 depends on light at all levels of protein synthesis regulation. In cyanobacteria, the control is carried out at the level of transcription, while in algae and higher plants, at the level of translation. D1 renewal is stimulated by inhibiting light (Aro et al. 1993).

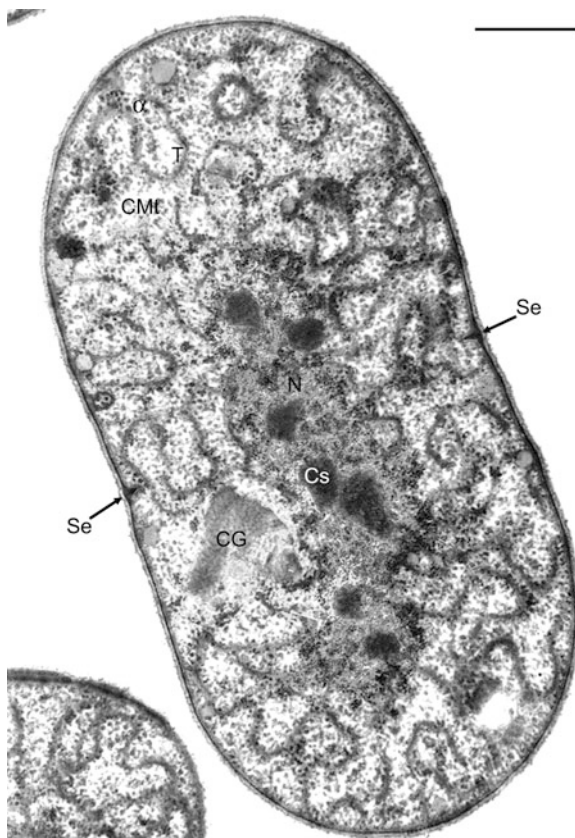
Fig. 2.14 Ultrastructure of a dividing *Chlorogloeopsis fritschii* ATCC 27193 cell in the culture grown in the glucose-supplemented medium under high light intensity (Baulina et al. 1982). α glycogen α -granules, ITS intrathylakoid space, Se septum, Sh sheath, T thylakoid. Scale bar 0.5 μ m



Study of the mutant strains demonstrated that protease FtsH, the key enzyme involved in the degradation of light-damaged D1 subunits, is localized in cyanobacterial thylakoid membranes, and not in CM. Thus, the enzyme plays a key role in renewal of the damaged photosynthetic apparatus of cyanobacteria (Mullineaux 2008; Komenda et al. 2006).

It may be assumed that the mechanisms of damage reparation under high light intensity function longer in *C. fritschii* than in the other two species. One more possibility to avoid total photodestruction of the cell components is probably the ability of the species to switch over to photo- or chemoheterotrophic growth. This assumption is based on the fact that in the presence of glucose under high light intensity *C. fritschii* proliferated actively while possessing a reduced thylakoid system, often swollen, and mass depositions of glycogen (Fig. 2.14). Cultivation of *A. variabilis* 458 in the presence of glucose and ribose contributed to resistance of the population to high-intensity light. Increased growth rate and elevated levels of all pigments were noted in the cells of such cultures (Suleimanova and Mineeva 1981). Under these conditions (in the presence of glucose), the cells containing larger amounts of storage substances, including glycogen, dominated in the population (Fig. 2.15). Most of the cells were dividing. In these cells, the cytoplasmic matrix was diluted, the thylakoids were highly curved and spread chaotically over

Fig. 2.15 Ultrastructure of a dividing *Anabaena variabilis* CALU 458 cell in culture grown in the glucose-supplemented medium under high light intensity (data obtained by Baulina, Suleimanova and Mineeva). α glycogen α -granules, CG cyanophycin granule, CMt cytoplasmic matrix, Cs carboxysome, N nucleoid, Se septum, T thylakoid. Scale bar 0.5 μ m

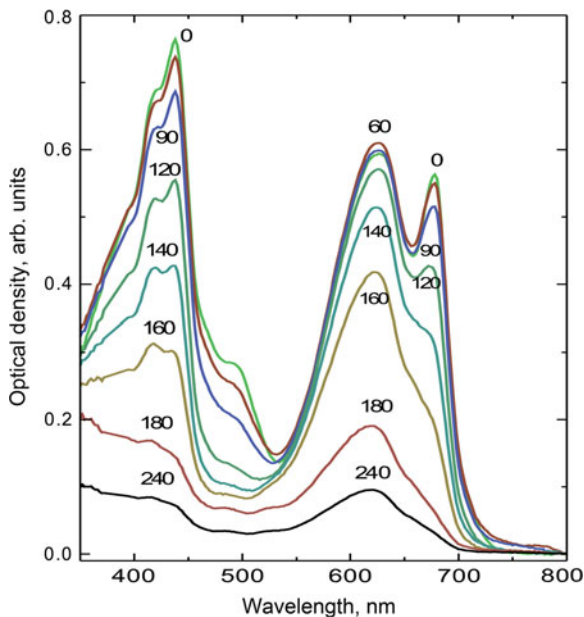


the cytoplasm, and the nucleoid was compact and located typically in the center of the cell. The photoheterotrophic *A. variabilis* 458 culture under high light intensity was heterogeneous in terms of resistance to photodamage: in some cells the typical picture of photooxidative destruction was observed. The response of the *Synechococcus* sp. 6301 culture to high light intensity in the presence of sugars was the same as when grown in the mineral medium.

2.2.3 Photodestruction of Cyanobacteria In Vitro

To reveal the differences in the levels of light sensitivity of the photosynthetic apparatus in various cyanobacteria species, experiments on irradiation of the cell suspensions with visible high-intensity light [5.5 mmol quanta/(m² s), that is, ~ 200 klx] were performed directly in spectrophotometric cuvettes without subsequent cultivation, that is, under conditions of limited opportunities for adaptive cell response through the regulatory repair mechanisms (Baulina et al. 2001,

Fig. 2.16 Changes in the absorption spectra of *Synechococcus elongatus* B-267 cell suspension during irradiation with high-intensity light (reprinted from Baulina et al. (2004) with kind permission from Pleiades Publishing, Ltd.) The numbers the exposure time (in minute)

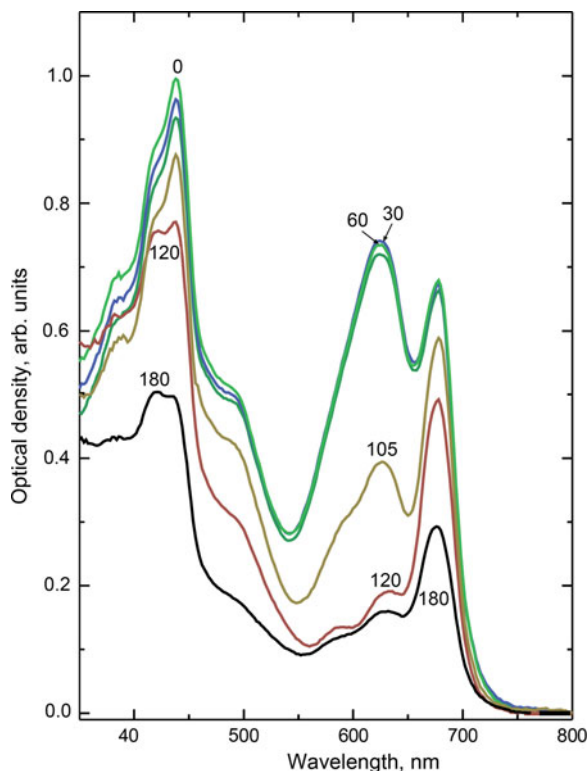


2004). The state of pigments and the ultrastructure determined immediately after irradiation were the criteria for damage evaluation. The same species were studied using strains *A. variabilis* 29413 and *S. elongatus*. The plan was to follow progressive stages of degradation of chlorophyll, carotenoids, and phycobilins in real time controlling the possible destructive changes in cells by electron microscopy. The experiments of this kind are necessary to investigate to which extent the changes induced by high light intensity correlate with the picture of the degradation of the cells of these cyanobacteria cultured in photooxidative conditions. Adequate description of the ultrastructural picture of photooxidative destruction should also contribute to the evaluation of the state of the population by the presence of irreversibly damaged and resistant cells under conditions of extreme changes in illumination conditions both under laboratory cultivation and in natural environment.

Presented below, result of our experiments are cited mainly from (Baulina et al. 2004).

Absorption spectra of the cell suspensions of *A. variabilis* 29413, *S. elongatus*, and *Synechococcus* sp. 6301 contained distinctive absorption bands of chlorophyll, phycobilins, and carotenoids, with respective maxima at 680, 626, and 480 nm (Figs. 2.16 and 2.17). The band with a maximum of ~ 420 nm caused by the presence of the products of chlorophyll photooxidation (Merzlyak et al. 1996) was also present. This figure also shows the spectral changes occurring during irradiation (absorption spectra for *Synechococcus* sp. 6301 are not shown because these changes were generally similar to those of *S. elongatus*). At the early stages of irradiation (10–30 min), an insignificant rise of phycobilins absorption maximum

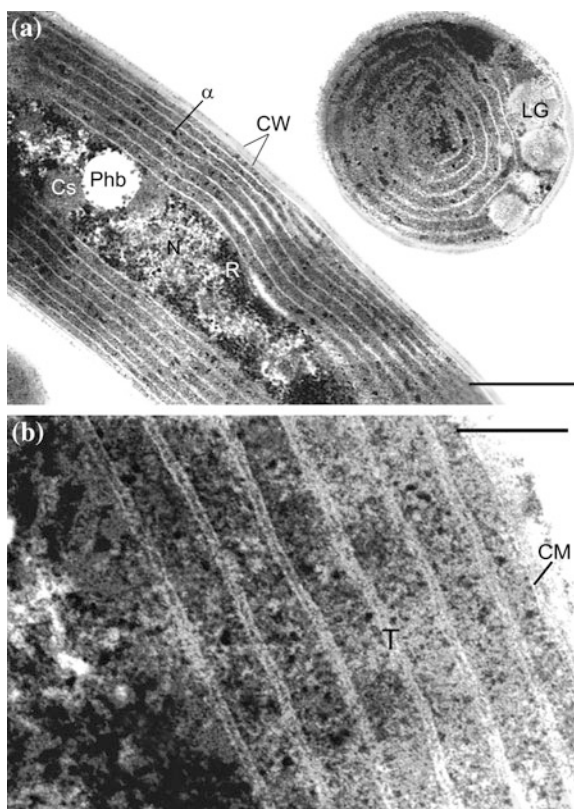
Fig. 2.17 Changes in the absorption spectra of *Anabaena variabilis* ATCC 29413 cell suspension during irradiation with high-intensity light (reprinted from Baulina et al. (2004) with kind permission from Pleiades Publishing, Ltd.) The numbers the exposure time (in minute)



was observed. As seen from figures, bleaching of the pigments in irradiated samples commenced after a certain lag period. Exposures to longer irradiation periods resulted in decreased optical density at the absorption bands of all pigments. Judging from the changes in optical density at 480 nm, the early stages of photodestruction of carotenoids in cyanobacteria occurred synchronously with chlorophyll degradation. At the terminal stages, only small quantities of carotenoids were retained in *S. elongatus*. In *A. variabilis* cells, the destruction of phycobilins proceeded faster than that of chlorophyll, and this trend persisted up to the final disappearance of phycobilins upon prolonged exposures. In *S. elongates* (as well as in *Synechococcus* sp. 6301), the presence of phycobilins was observed even after complete bleaching of chlorophyll.

Morphology and ultrastructure of *S. elongates* were typical for this genus (Fig. 2.18a). As a rule, in the plane of the section, five to six thylakoids with a structure typical of cyanobacteria—two closely appressed membranes with three-layer profile—surrounded the central part of the cell (Fig. 2.18b). The cytoplasm was dense, so that the phycobilisomes located at the outer surface of the thylakoids were not resolved. The spaces between thylakoids contained occasional α -granules of glycogen and moderately dense granules of presumably lipid origin. The ribosomes occupied all the space between the internal thylakoid and a thin-fibrillar

Fig. 2.18 Ultrastructure of *Synechococcus elongatus* B-267: general view (a) and the thylakoid region (b) (reprinted from Baulina et al. (2004) with kind permission from Pleiades Publishing, Ltd.) α glycogen α -granules, *Cs* carboxysome, *CW* cell wall, *LG* lipid granules of moderate electron density, *N* nucleoid, *Phb* poly- β -hydroxybutyrate granules, *R* ribosomes, *T* thylakoid. Scale bar a 0.5 μ m, b 0.1 μ m



nucleoid. In the nucleoid area, there were carboxysomes and structures similar to granules of poly- β -hydroxybutyrate (see Fig. 2.18a).

During the lag period preceding the pigment bleaching (60 min), the ultrastructure of the majority of the *S. elongatus* cells was fully similar to that of the intact cells. At the same time, during this period, some cells already exhibited destructive changes in the thylakoids, cytoplasmic matrix, and nucleoid. Upon irradiation for 180 min, modified cells were abundant in the suspension (Fig. 2.19). The photosynthetic membranes in such cells lost their three-layer profile. The thylakoids were seen as fragmented parallel lines of moderate electron density. At the same time, destructive changes of the CM were usually not discernible in these cells. The cytoplasmic matrix became homogeneous, with fine grains uniformly distributed between poorly visible contours of the thylakoid membranes. The nucleoid zone visible on the section (with peripheral ribosomes) was significantly reduced, and the DNA strands adhered to each other. The carboxysomes and α -granules did not show conspicuous changes. After irradiation for 240 min, no undamaged cells were observed in the suspensions. Nevertheless, throughout the period of observations, we did not reveal the cells with pronounced signs of autolysis. In addition to the alterations described above, upon long

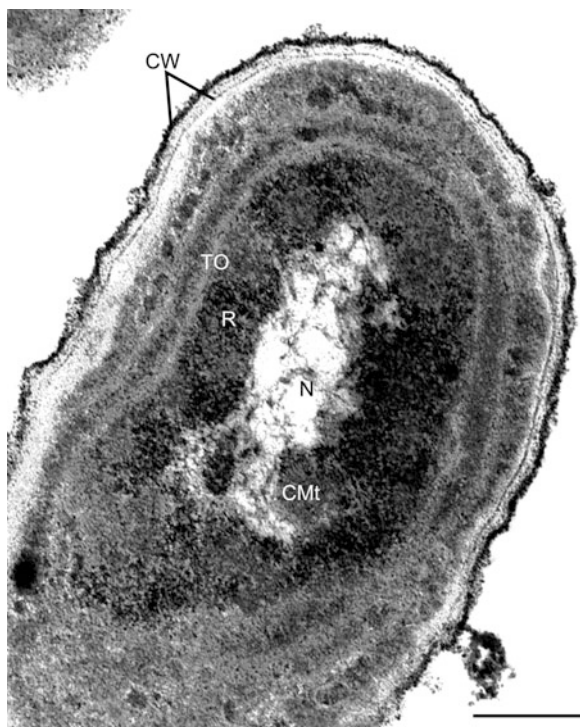
Fig. 2.19 Ultrastructure of *Synechococcus elongatus* B-267 after 180 min irradiation of cell suspension with high-intensity light (reprinted from Baulina et al. (2004) with kind permission from Pleiades Publishing, Ltd.) α glycogen α -granules, *CM* cytoplasmic membrane, *N* nucleoid, *Pg* peptidoglycan, *R* ribosomes, *TO* outlines of the thylakoids. Scale bar 0.2 μ m



irradiation we observed ultrastructural changes in the outer membrane of the cell wall. In most cases, this membrane lost its contact with peptidoglycan and acquired an atypical nonuniform wave-curved profile. The contours of this membrane were not clearly visualized, because its surface was masked by electron-dense depositions similar to the glycocalix material. The examination of ultrathin sections did not reveal release of the degradation products into the periplasmic space.

In the case of both *Synechococcus* sp. 6301 and *S. elongatus*, the cells with evident signs of destruction were observed in the initial period of pigment degradation. The ultrastructural changes in both species were generally similar. Similar to *S. elongatus*, the thylakoid membranes lost their three-layer profile but were seen as lines of low electron density (Fig. 2.20) or two parallel lines of moderate electron density. In *Synechococcus* sp. 6301, other ultrastructural changes were as follows. At the late stages of irradiation, CM was usually not observed, while the cell wall looked undamaged. In the central part of the cell, where the destruction signs were most evident, there were ribosome aggregates

Fig. 2.20 Ultrastructure of *Synechococcus* sp. PCC 6301 after 360 min irradiation of cell suspension with high-intensity light (reprinted from Baulina et al. (2004) with kind permission from Pleiades Publishing, Ltd.) *CMt* cytoplasmic matrix, *CW* cell wall, *N* nucleoid, *R* ribosomes, *TO* outlines of the thylakoids. Scale bar 0.2 μ m

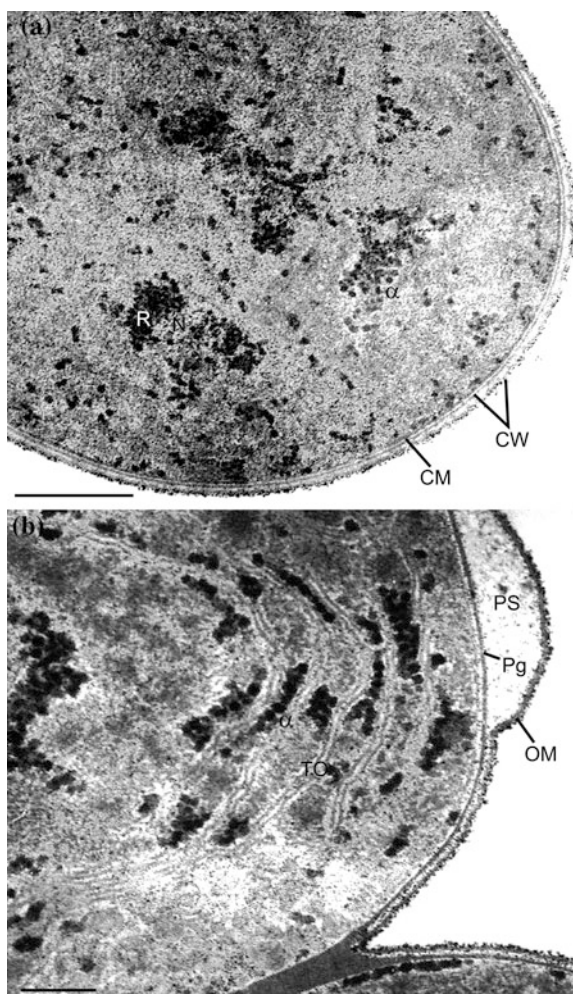


separated by regions of homogenous cytoplasmic matrix similar in electron density to that of the thylakoids. Neither *Synechococcus* sp. 6301 nor *S. elongatus* showed any sign of cell autolysis. Thus, the impact of irradiation on *Synechococcus* sp. 6301 involved not only thylakoids, but also the cytoplasmic matrix and nucleoid, similar to the previously described species, and affected also the CM. It should be noted that, even after prolonged irradiation of *Synechococcus* sp. 6301, apart from the cells with destructive changes, intact-looking cells occurred frequently in the suspensions.

The ultrastructure of *A. variabilis* 29413 was typical of this species grown under optimal conditions and similar to that of *A. variabilis* 458 (see Fig. 2.3). The thylakoids were seen as two closely appressed membranes with three-layer profile, although in some cells they were located at a distance of ~ 20 nm. They occupied the major part of the cytoplasm and formed numerous bendings and concentric circles. In many cases, the neighboring thylakoids were positioned in parallel; occasionally, there were aggregates of closely connivent thylakoids. Within the cells there were distinguished zones of the nucleoid with peripheral aggregates of ribosomes, the carboxysomes, and glycogen depositions (α -granules). The surface of the outer membrane contained depositions of finely dispersed capsular material.

Already after 30 min of irradiation, no structurally intact thylakoids were found on ultrathin sections (Fig. 2.21a). The thylakoid membranes lost their distinct

Fig. 2.21 Ultrastructure of *Anabaena variabilis* ATCC 29413 after 30 min (a) and 180 min (b) irradiation of cell suspension with high-intensity light (reprinted from Baulina et al. (2004) with kind permission from Pleiades Publishing, Ltd.) α glycogen α -granules, CM cytoplasmic membrane, CW cell wall, N nucleoid, OM outer membrane, Pg peptidoglycan, PS periplasmic space, R ribosomes, TO outlines of the thylakoids. Scale bar a 0.5 μ m, b 0.2 μ m



three-layer ultrastructure; the thylakoid contours became almost indistinguishable from the adjacent cytoplasm. The observed picture was similar to the one presented in Fig. 2.6. Occasional cells (data not shown) on the same sections contained a granular background of the cytoplasmic matrix and clearly visible electron-transparent winding lines that corresponded in thickness and position to the thylakoids of the intact cells. In the immediate proximity to these lines, low-electron-density granules of presumably lipid origin were located; these granules probably resulted from the degradation of thylakoids (de Vasconcelos and Fay 1974). Similar to the intact cells, the spaces between destroyed thylakoids accommodated a compact nucleoid zone, ribosomes, and α -granules (Fig. 2.21a). The CM and the cell wall also appeared intact. Upon prolongation of irradiation exposure to 60 min, we did not observe additional changes in cell ultrastructure.

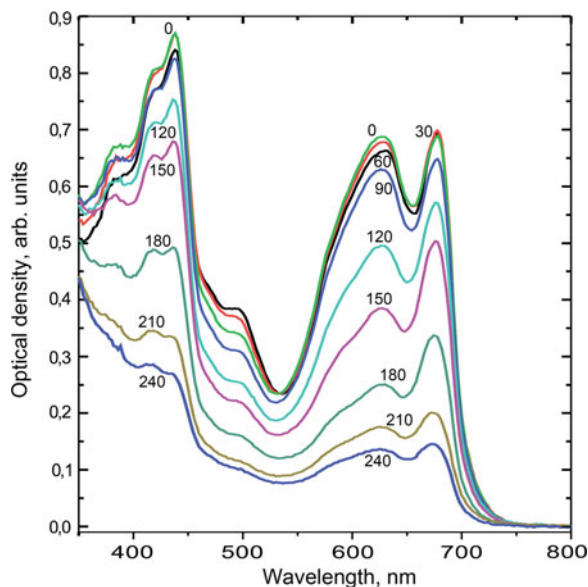
Thus, the disturbance of the structural organization of the thylakoid membranes occurred in parallel with retention of the spectral characteristics of all types of pigments at the same level as in the intact cells.

Dramatic destructive changes in the ultrastructure of *A. variabilis* were observed after prolonged irradiation (Fig. 2.21b): the content of chlorophyll and carotenoids was drastically reduced, and phycobilins were almost fully destroyed (Fig. 2.17). The ultrastructural changes after exposures for 120 and 180 min were principally similar. The cytoplasm appeared as irregularly spaced clots of various electron densities. The nucleoid zones, the ribosome clusters, and the carboxysomes were not revealed. The “ghosts” of the thylakoid membranes were distinctly seen within the cytoplasmic matrix of nonuniform density. On the sections, these ghosts had an appearance of irregular bended lines, often organized in pairs. Granules similar to glycogen particles occupied the interstices between the cytoplasmic clots and degrading thylakoids. In such cells, CM was destroyed, and the peptidoglycan layer acquired a finely grained structure. The outer membrane retained its ultrastructural integrity and three-layer profile, but it produced protruberances at small regions of the cell surface. Further on, these protrusions apparently transformed to separated vesicles. The capsular material had the same appearance as in the intact cells. In many cases, the three-layer profile of the outer membrane was quite obvious in the separated vesicles. Thus, the prolongation of light exposure promoted the alteration of the outer membrane binding to the peptidoglycan layer; this tendency became evident already after 30 min of light exposure. The peptidoglycan layer turned loose, while retaining its rigidity. The space between this layer and the protrusions of the outer membrane, as well as the rest of the periplasmic space were filled with a finely dispersed granular material containing presumably the destructed components of the cytoplasm (see Fig. 2.21b). Even after long irradiation, we observed neither disruptions of the outer membrane nor the leakage of electron-dense products of lysis into the medium. At these stages, the populations occasionally contained cell remnants with fully lysed content and still unbroken bounding layer that was formed by the structurally modified cell wall. In the structure of this bounding surface layer, the outer membrane and the peptidoglycan layer were indistinguishable.

It should be noted that ultrastructural changes in the cell population of *A. variabilis* 29413 at different stages of irradiation (30, 60, 120, and 180 min) were comparatively uniform. However, even after long exposure to light, we occasionally observed the cells retaining the ultrastructure characteristic of short irradiation periods. At the same time, after long irradiation we could not observe cells with structurally intact thylakoids.

In contrast to *A. variabilis* 29413, the original (control) *C. fritschii* culture, as it is typical of the species, was heterogeneous in cell morphology and ultrastructure. Irradiation during 60 min did not affect the ultrastructure; this result correlated with the practically undamaged state of the pigments during this period (Fig. 2.22). Moreover, cells similar in organization to the control ones, predominated after 120–150 min exposure and were present in the population after 210–240 min irradiation. After 120–150 min of irradiation, together with

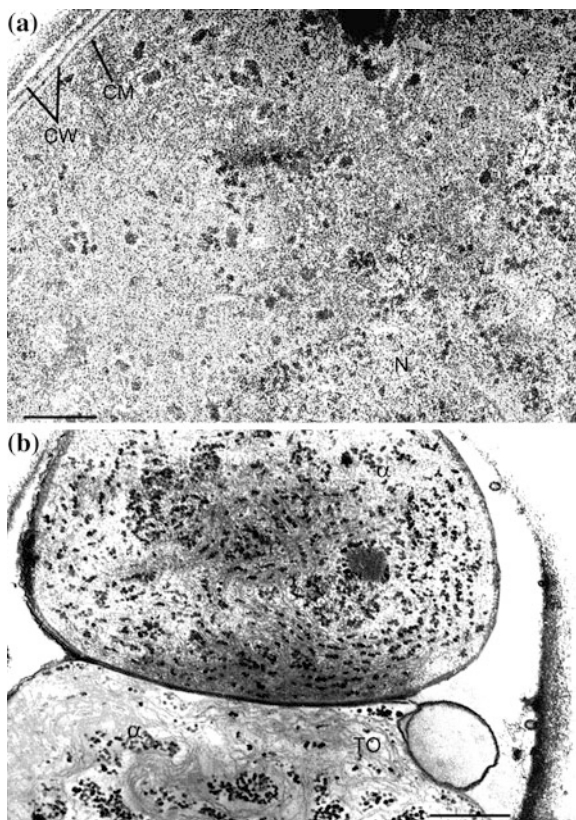
Fig. 2.22 Changes in the absorption spectra of *Chlorogloeopsis fritschii* ATCC 27193 cell suspension during irradiation with high-intensity light (data obtained by Chivkunova, Merzlyak and Baulina). The numbers the exposure time (in minute)



increasing bleaching of pigments (Fig. 2.22), the cells with destructive changes in the thylakoid membranes were revealed in the population, although the cell wall, CM, and nucleoid retained their integrity (Fig. 2.23a). In the case of 210–240 min exposure, destructive changes in these cells increased: CM was being destroyed and granules similar to the lipid ones appeared. In some cells, the peptidoglycan layer was lost, thylakoids turned to disordered accumulations of membrane debris, the cytoplasm matrix underwent autolysis, the nucleoid, carboxysomes, and granules of poly- β -hydroxybutyrate could not be revealed, while the granules of glycogen were present in abundance (Fig. 2.23b). In the case of these cells, similarity between the ultrastructural pictures of cell destruction in two species, *C. fritschii* and *A. variabilis* 29413, should be noted, with the modifications being revealed earlier and developing to a greater extent in the latter strain.

The results presented in this section (Baulina et al. 2004) suggest a conclusion that the pattern of photodestructive processes in the examined cyanobacterial species is complicated; this is clearly manifested in diverse distortions of the membrane structure and cell integrity. A characteristic feature of these distortions is dramatic changes in the ultrastructure of thylakoids. The lack of a three-layer profile of the thylakoid membranes, which is typical of the elementary membrane, which was revealed by TEM, points to the impairment of the phospholipid bilayer in consistency with the conclusions reached by other authors (Schmetterer et al. 1983). It is noteworthy that the destructive alterations in the ultrastructure of thylakoid membranes in *A. variabilis* 29413 were observed already during the lag period preceding bleaching of chlorophyll, carotenoid, and phycobilins. On the other hand, in *Synechococcus* species and *C. fritschii*, the ultrastructural changes occurred simultaneously with the destruction of all pigments. In the case of

Fig. 2.23 Ultrastructure of *Chlorogloeopsis fritschii* ATCC 27193 after 150 min (a) and 120 min (b) irradiation of cell suspension with high-intensity light (data obtained by Baulina, Chivkunova, Merzlyak). α glycogen α -granules, *CM* cytoplasmic membrane, *CW* cell wall, *N* nucleoid, *TO* outlines of the thylakoids. Scale bar **a** 0.2 μ m, **b** 0.5 μ m



A. variabilis 29413, the initial damage to the thylakoid membranes was followed by a cascaded amplification of destructive changes in other cell structures.

Apart from destruction of the thylakoids, in all species under study, homogenization of the cytoplasm, and degradation of the nucleoid were observed, although carboxysomes still persisted in the nucleoid zone. In *A. variabilis* 29413 and *Synechococcus* sp. 6301, the disturbances of CM ultrastructure progressed up to full degradation of this membrane. The outer membrane of *A. variabilis* 29413 retained for a long time the ultrastructure typical of an elementary membrane and showed extensibility in local regions. The capacity for extension was also observed in *S. elongatus*, although to a much lesser extent. By the end of the irradiation period, destructive processes in the cytoplasm apparently occurred in all the cells in the population of these two species, so that undamaged cells were not revealed. In *A. variabilis* 29413 some cells completely lost their cytoplasmic content. It is conceivable that destruction of the cytoplasm in this case could be caused not only by direct action of irradiation but also by the activity of hydrolytic enzymes released from the periplasmic space due to impaired integrity of the CM. The products of cytoplasm degradation apparently penetrated through the

peptidoglycan gel which, judging from its altered ultrastructure, became more permeable to macromolecules.

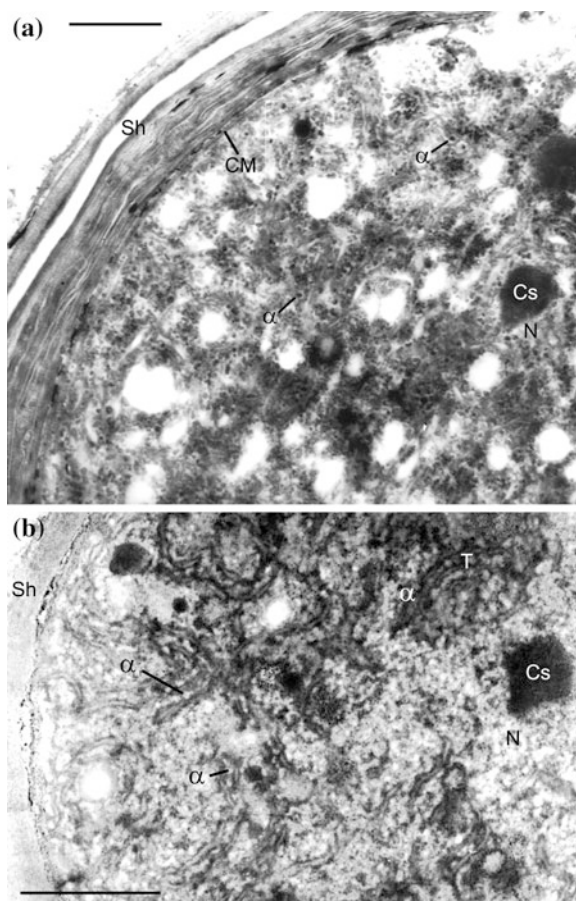
The comparison of responses of cyanobacteria to the short-term action of extremely high-intensity light (200 klx) and the behavior of the same organisms during cultivation under high light intensity (9–10 klx) revealed a certain similarity in the ultrastructural manifestations of cell destruction. Exposure of *Synechococcus* sp. 6301 to both treatments resulted in destruction of the thylakoids and CM, homogenization of the cytoplasm, and degradation of the nucleoid, with the retention of carboxysomes in the nucleoid zone. At the same time, the cell wall remained unchanged, and cell lysis did not occur; at late stages, many cells in the population were similar to intact cells in terms of their ultrastructure. When *A. variabilis* 458 was grown at a high light intensity, similar destructive changes took place at shorter exposures to irradiation, and many cells were lysed at later stages. Upon culturing of this cyanobacterial species under extreme illumination conditions, the thylakoids became vesiculated at early stages of irradiation and were destroyed at late stages. The outer membrane was found to be the most resistant cell structure. On the other hand, the peptidoglycan layer of the cell wall lost its rigidity and became perforated with numerous pores and narrow canaliculi. Similar changes were also characteristic of the cells of *A. variabilis* 29413 irradiated with high-intensity light for short periods. The most significant distinction was that no vesiculation of the thylakoids was observed in *A. variabilis* 29413. Both under physiological conditions and during irradiation of cell suspensions with high-intensity light, cyanobacteria of different genera exhibited destructive changes of variable degrees: both strains of *A. variabilis* were more sensitive to light treatment than *Synechococcus* species and *C. fritschii*. As for the latter species, only irradiation of the suspension with light of high intensity demonstrated that this cyanobacterium was also susceptible to photooxidative destruction, although no destructive processes could be revealed upon cultivation under high light intensity during the period of observation.

It appears that the pattern of ultrastructural changes occurring in cyanobacteria under photooxidative conditions are affected by the regulatory mechanisms of cell response and by species-specific differences in the chemical composition and macromolecular organization of the thylakoid membranes, CM, cell wall, and other cell components accounting for the differences in their structural stability (Baulina et al. 2004). In general, the described picture of destructive changes in cyanobacteria indicates a more complex nature of photooxidative damage than it was considered previously.

2.2.4 Ultrastructure of *Chlorogloeopsis fritschii* ATCC 27193 Grown in the Dark

Genetically determined metabolic flexibility of *C. fritschii* is responsible for its capacity for chemoheterotrophic growth in the dark in the presence of exogenous sources of carbon and energy (Fay 1965). In this section, the results of studies on

Fig. 2.24 Ultrastructure of *Chlorogloeopsis fritschii* ATCC 27193 in the culture growing in the dark in the presence of glucose: ultrathin sections contrasted with lead citrate (a) and uranyl acetate (b). α glycogen α -granules, CM cytoplasmic membrane, Cs carboxysome, N nucleoid, Sh sheath, T thylakoid. Scale bar a, b 0.5 μ m



the ultrastructure of this cyanobacterium grown in the presence of glucose are presented (Baulina et al. 1978). It should be noted that even in the presence of sugars growth in the dark was slow, the cells were typically individual and surrounded by thick multilayer sheaths (Figs. 2.24 and 2.25).

As it will be demonstrated in this and the following chapters, cyanobacterial sheaths and other external surface structures of similar chemical nature and functions, i.e., capsules, depositions of amorphous mucus, and mucous thin covers over groups of cells, all play an important role in the adaptation processes developing in the cells and populations of these microorganisms. In the case of comprehensive analysis of experimental data, it seems appropriate to group all these structures under a common term mucous surface structures (MSS) in contrast to the widely used term glycocalyx. The latter term was initially used in description of eukaryotic organisms and, to our mind, is not accurate in description of prokaryotes, whose cell envelope organization is totally different comprising a

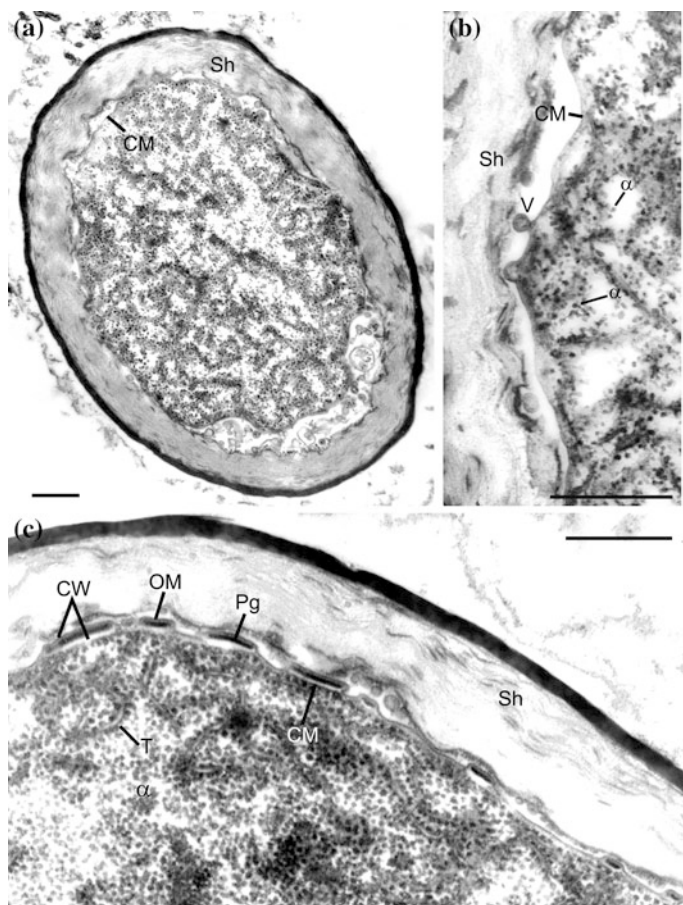


Fig. 2.25 Ultrastructure of *Chlorogloeopsis fritschii* ATCC 27193 in the culture growing in the dark in the presence of glucose: a protoplast surrounded with a sheath (a), formation of CM vesicles in the protoplasts (b), changes in structure of the cell wall (c) (Baulina et al. 1978). α glycogen α -granules, CM cytoplasmic membrane, CW cell wall, OM outer membrane, Pg peptidoglycan, Sh sheath, T thylakoid, V vesicle. Scale bar a, b, c 0.5 μ m

peptidoglycan layer, a periplasmic space, and an outer membrane. The only exception is prokaryotes lacking cell wall, that is mycoplasmas and some archaea.

Laminated MSS of cyanobacteria, often of complex structure, are typically called sheaths. They may differ in the number and volume of the layers, as well as in their chemical features, packing and orientation of the polymers comprising each layer. To define the individual layers with polymeric fibril orientation perpendicular to the cell wall, a number of researchers use the term capsule, common for all bacteria. Multilayered sheaths, including those comprising homogenous electron-dense layers, in addition to the fibrillar ones, and lack covalent bonds with the cell wall outer membrane, are common in *C. fritschii* and members of the

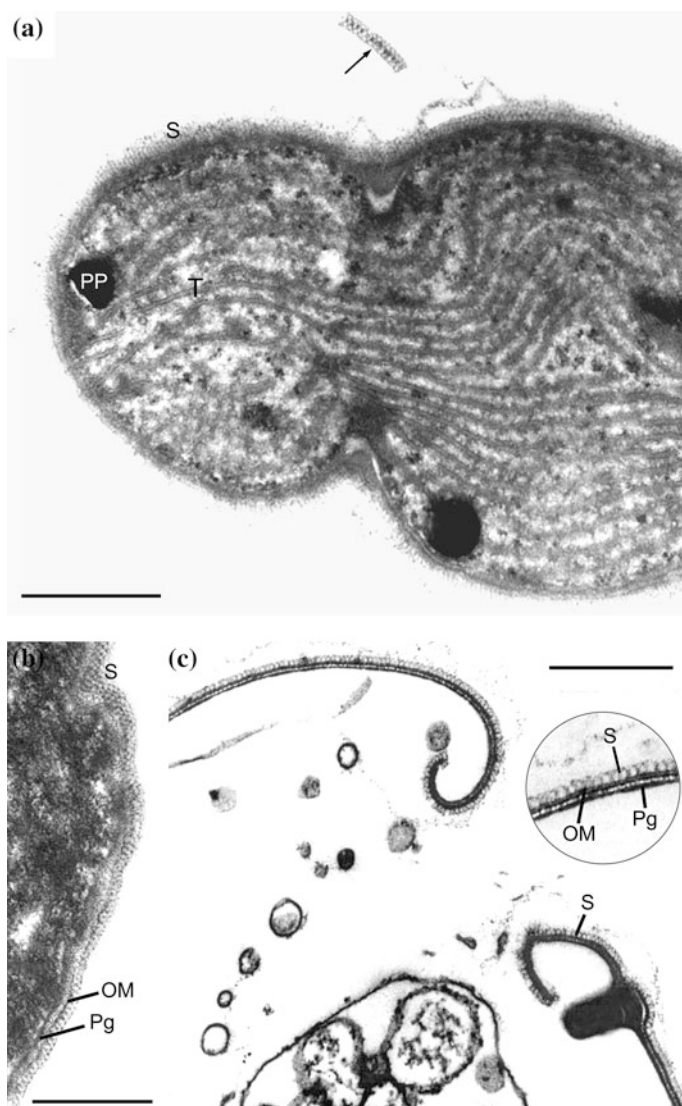
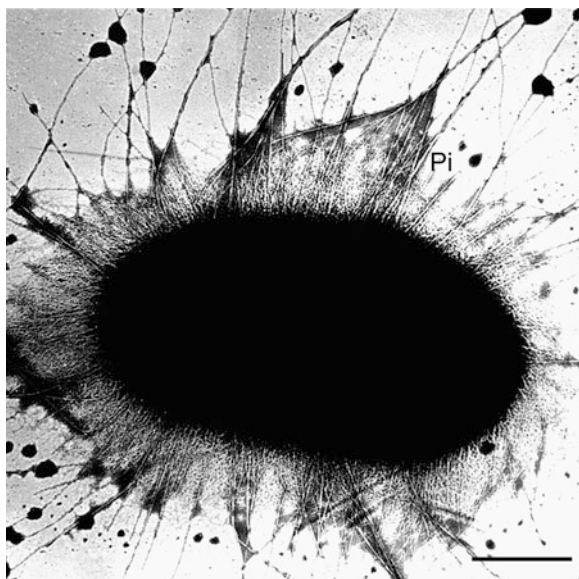


Fig. 2.26 Ultrastructure of *Synechocystis aquatilis* CALU 428 general view of a dividing cell (a), cell region with the envelope (b), envelope of an autolysed cell (c). OM outer membrane, Pg peptidoglycan, PP polyphosphate granules, S S-layer, T thylakoid. The arrow indicates an peculiar paracrystalline structure. Scale bar a, b, c 0.5 μm

genus *Gloeocapsa*, *Gloeotheca*, *Gloeobacter* (Rippka et al. 1979), *Fischerella* (Pritzer et al. 1989), *Nostoc* (Hill et al. 1994). For *Nostoc commune*, the outer layer of the sheath was shown to be enriched with calcium, silicon, and magnesium (Hill et al. 1994). Electron-dense layers may contain proteinaceous components

Fig. 2.27 *Synechocystis aquatilis* CALU 428 with peritrichous pili. Negative staining with phosphotungstic acid, TEM. *Pi* pili. Scale bar 1 μm



(Jürgens and Weckesser 1985; Baulina et al. 2008). Data on the chemical composition of cyanobacterial MSS are presented in greater detail in Sect. 5.1.1.

For a reader getting the first acquaintance with the diverse world of cyanobacteria, it should be specified that MSS, such as capsules or sheath, are characteristics of most, although not all species of these microorganisms. In many, other structures called S-layers, typical of most prokaryotes and comprising protein or glycoprotein surface layers (see Figs. 2.1 and 2.26), are located above the cell wall outer membrane (Jensen and Sicko 1972; Lounatmaa et al. 1980; Vaara 1982; Gromov 1986; Mamkaeva 1986; Sleytr et al. 1994; Hoiczky and Hansel 2000; Šmarda et al. 2002). In cyanobacteria with capsules and sheath, S-layers may also be present. Bacterial S-layers possess numerous functions such as adhesive, protective, and barrier ones, including those of adaptive importance (Sára et al. 1990). While the functions of these structures in cyanobacteria are studied less, their role in the processes responsible for gliding in a number of filamentous cyanobacterial species (Hoiczky and Baumeister 1995) and swimming of a unicellular *Synechococcus* sp. WH8102 (McCarren et al. 2005) has been established. Besides, cyanobacterial S-layers act as a matrix for formation and deposition of calcium, magnesium, and strontium minerals (Schultze-Lam and Beveridge 1994a).

In our study of the cyanobacterial species *Synechocystis aquatilis* Sauv. CALU 428 (referred to as *Synechocystis aquatilis* hereinafter), S-layers were found to consist of hexagonally packing subunits, often of uneven thickness (Fig. 2.26a and b) and may be peeled off cell surface. The latter fact indicates the lability of these structures. At the same time, upon autolysis these layers retain their intact ultra-structure and connection to the cell wall outer membrane (Fig. 2.26c). This interesting phenomenon was revealed in other bacteria, for example, in *Flexibacter*

polymorphus (Ridgway et al. 1975). For cyanobacteria of genus *Synechococcus* (strain GL24), high resistance of the S-layer complex and the underlying cell wall layers in the absence of cell lysis upon treatment with the structure-breaking chemical agents is considered to be connected with the extreme conditions of the natural habitat of the microorganism (Schultze-Lam and Beveridge 1994b).

On the surface of a number of unicellular and filamentous cyanobacteria, pili (fimbriae) are localized (Ratner et al. 1976; Dick and Stewart 1980; Vaara 1982; Johansson and Bergman 1994; Adams and Duggan 2008) (Fig. 2.27; see also Fig. 2.1). These are fibrillar accessory structures characteristic of prokaryotes built of protein subunits packed in cylinders. Various types of spines also occur on the surface of cyanobacterial cells (see Fig. 2.1; see also Fig. 4.8d and references in Chap. 4). Their function is not studied yet. Concerning the pili, which are present in *Synechocystis aquatilis* (Fig. 2.27), as well as the S-layers, it should be noted that for *Synechocystis* sp. PCC 6803, another representative of this genus, involvement of these structures, particularly the pili of type IV, in the twitching motility has been established (Bhaya et al. 2000). In general, bacterial pili of various types (I–IV) are involved in cellular adhesion, motility, and conjugation process (Pinevich 2006).

Going back to description of *C. fritschii* growing in the dark, it should be noted that the fact of predomination of unicellular forms is in accordance with the data on suppressed formation of filamentous forms under conditions of photoheterotrophic and chemoheterotrophic growth (Evans et al. 1976; Evans and Britton 1983). On the basis of these data, the authors concluded that emergence of various morphological cell types in a *C. fritschii* culture was a consequence of external factors.

Upon switching to chemoheterotrophic growth, the ultrastructural plasticity of the species was revealed to its full extent at both subcellular and cellular levels. The thylakoid system was reorganized, resulting in the changes of thylakoid spatial arrangement in the cytoplasm matrix, shortening of the membrane contours in the section plane, and inability to reveal their three-layer ultrastructure (see Fig. 2.24). Different changes in the thylakoid system were observed in *A. variabilis* 29413 growing in the dark in the presence of fructose (Pinevich and Volkov 1988). In this latter case, curved, but not shortened thylakoids filled the whole cytoplasm. Analysis of the pigment composition and a number of other parameters suggested that the changes in the photosynthesis apparatus were of adaptive nature. At the same time, low electron density of the cytoplasmic matrix is typical of this species as well as of the chemoheterotrophic *C. fritschii* culture under study. In the cells of the latter one, the cytoplasm was filled with α -granules of glycogen of atypical spherical shape (they are typically ellipsoid in cyanobacteria). The fact that these granules were filled with neutral polysaccharides was confirmed by differential staining of ultrathin sections with lead citrate according to Reynolds (Reynolds 1963) (Fig. 2.24a) and uranyl acetate (Fig. 2.24b) upon fixation with glutaraldehyde and osmium tetroxide. In the first case, glycogen granules appeared electron dense, while in the second case, they remained electron transparent since uranyl acetate does not interact with neutral polysaccharides (Geyer 1973).

In the early studies, similar data on *C. fritschii* ultrastructure upon long-term cultivation in the dark in the medium with sucrose, including the data on spatial arrangement of the thylakoid system and accumulation of numerous glycogen granules, were obtained using the preparations fixed with KMnO_4 which does not preserve proteins and nucleic acids (Peat and Whitton 1967). The connection between glycogen deposition in the cells of various cyanobacterial species and the heterotrophic type of their constructive metabolism was noted by a number of researchers (Rippka 1972; Raboy et al. 1976). The direction of biosynthetic processes in *Aphanocapsa* sp. 6714 has been studied in detail depending on the type of constructive and energy metabolism (Pelroy et al. 1976). Determination of the activity of the synthesis of major cell polymers (proteins, lipids, RNA, and glycogen) revealed that growth of this cyanobacterium in glucose-containing medium in the dark (chemoheterotrophic growth) or in the light (photoheterotrophic) in the presence of a photosynthesis inhibitor Diuron led to significant decrease in the rates of lipid, RNA, and protein syntheses. The main substance synthesized under these conditions was glycogen. In the experiments on *C. fritschii* growth in the dark reported in this section, as well as in the experiments with this species and *A. variabilis* 458 grown under high light intensity in the presence of sugars (see Sect. 2.2.2), similar processes probably occurred. This is confirmed by a decrease in the electron density of the cytoplasmic matrix, the absence of ribosome aggregations, inability to detect the characteristic nucleoid zones in many of thin sections, and, on the contrary, abundance of glycogen α -granules.

The spectacular feature of most of the cells in a population at the stage of growth (day 15) was reorganization of the cell wall up to complete reduction of its components revealed by electron microscopy, that is, destruction of the outer membrane and the peptidoglycan layer and formation of protoplasts (see Fig. 2.25a). At the same time, the cells in the studied preparations always contained a complex sheath. CM of the protoplasts formed characteristic protrusions which subsequently formed vesicles localized between the protoplast surface and the sheath (see Fig. 2.25b). In the above-cited literature (Peat and Whitton 1967), the absence of the cell wall could be seen in some images, although this fact was not discussed in the text. Moreover, while there are indications by other authors that protoplasts enclosed in sheaths are present in *C. fritschii* culture upon growth under low-intensity illumination and at 45 °C (above the optimal temperature), the phenomenon of the absence of the cell wall in cyanobacteria is not discussed (Findley et al. 1970). It should be noted that *C. fritschii* protoplasts are sometimes revealed in the stationary-phase culture growing under optimal light and temperature conditions (Gusev et al. 1982). Cyanobacteria *N. muscorum* CALU 304 was also able to form the protoplasts producing surface vesicles upon growth under optimal conditions. In this case, however, the cells tended to form constrictions and divide; chains of 2–5 protoplasts were detected (Gorelova 2001). To the best of our knowledge, there is no other data on spontaneously formed protoplasts or spheroplasts in laboratory cultures of free-living cyanobacteria.

Observation of numerous protoplasts in *C. fritschii* culture suggested a hypothesis that at a definite stage of growth in the dark, cells of this cyanobacterium are

L-forms which are characterized by the presence of a multilayer sheath probably performing some of the cell wall functions and facilitating protoplast protection against osmotic shock (Baulina et al. 1978; Gusev et al. 1982). It is interesting, that protective function of the colanic acid capsule established in the study of L-form-like growth *Escherichia coli* K-12 (Joseleau-Petit et al. 2007).

In microbiological literature, the term L-forms is used to define bacteria capable of growth in the absence of a rigid cell wall (Prozorovsky et al. 1981; Domingue and Woody 1997). In cyanobacteria, the ability for L-transformation and induction of L-form-like cells was experimentally demonstrated only in the early 1980s by the researchers of the group headed by Professor Mighael V. Gusev, at the Faculty of Biology, Moscow State University (Gusev et al. 1981, 1983). These studies are reported in Chap. 3.

Determination of the ability of darkness-induced *C. fritschii* protoplasts to reverse to original cells is an unsolvable problem for an individual cell form. It is known from the literature that the capacity for reversion depends on the ability to reconstruct the cell wall with the obligatory presence of macromolecular precursors of peptidoglycan and components of the outer membrane. As a rule, the protoplasts of gram-positive bacteria, spheroplasts of gram-negative bacteria, and unstable L-forms can be reversed easily (see Chap. 3). Therefore, to determine the possibility of reversion for at least some L-form-like variants of *C. fritschii*, ultrastructural evidence of synthesis of peptidoglycan and cell wall outer membrane should be collected. This issue is presently far from resolution. However, there is evidence that many cell forms in dark-growing *C. fritschii* cultures were at the stage of cell wall resynthesis: discontinuous peptidoglycan layer and alternating regions with complete cell wall, including the outer membrane, and those with no cell wall, are characteristic of these cells (Fig. 2.25c). Systematic studies performed on pathogenic bacteria at the Gamaleya Research Institute of Epidemiology and Microbiology (Russian Academy of Medical Sciences) demonstrated that discontinuous cell wall was only observed during the early stage of reversion (Prozorovsky et al. 1981). This conclusion, as well as our data (Fig. 2.25c), agree with the results of the later study of *E. coli*, which established that L-form-like growth required ongoing peptidoglycan synthesis and hypothesized that all L-forms have possibly residual peptidoglycan synthesis (Joseleau-Petit et al. 2007).

Thus, formation of protoplasts in *C. fritschii* culture upon changes in the growth conditions is a manifestation of its ultrastructural plasticity at the cellular level indicating the possible L-transformation under conditions of growth in the absence of light.

2.3 Concluding Remarks

Results reported in this chapter allow for the following conclusions. Among the representatives of three cyanobacterial genera, *Synechococcus*, *Anabaena* and *Chlorogloeopsis*, differing by the degree of obligate phototrophy upon cultivation

under varying light regimes, various forms of ultrastructural plasticity were revealed at subcellular, cellular, and population levels. These include:

Subcellular level

- (1) Changes in configuration of the thylakoids reflected in a considerable separation of the constituent membranes with the preservation of their ultrastructural integrity in the obligate phototroph *A. variabilis* 458 upon long-term cultivation in the dark. In the case of transfer to optimal illumination conditions after a limited term of incubation in the dark, this phenomenon is reversible and is accompanied by the preservation of cell viability.
- (2) Formation of vesicles by CM of the *C. fritschii* protoplasts upon growth in the dark.
- (3) Ultrastructural changes of the cell wall peptidoglycan layer in dark-growing *C. fritschii*.

Cellular level

- (1) The absence of glycogen in *A. variabilis* 458 cells due to its consumption as a carbon and energy source in the dark-incubated culture and, on the contrary, its presence in the culture returned into the phototrophic conditions. Predominant (compared to other intracellular structures) glycogen accumulation in photoheterotrophic cells of *A. variabilis* 458 and *C. fritschii* and in chemoheterotrophic cells of *C. fritschii*.
- (2) Complete reduction of the cell wall in *C. fritschii* growing in the dark (formation of protoplasts).

Population level

Changes in the population structure. Heterogeneity of the cells in populations of all studied species by (a) ultrastructural manifestation of photooxidative destruction of the thylakoids and other cellular components and (b) the degree of changes in the cell wall ultrastructure.

Experiments on the damaging effects of high-intensity light on the ultrastructure of cyanobacteria demonstrated that in *A. variabilis* strains, all cell structures (except for cyanophycin granules) including thylakoids, CM, peptidoglycan layer, and cell wall outer membrane, are subjected to destruction much faster than in *C. fritschii*, *Synechococcus* sp. 6301, and *S. elongatus*. In the two latter species, ultrastructural changes which could be attributed to manifestations of the ultrastructural plasticity, such as increased intrathylakoid space, were not registered. On the contrary, at least in *Synechococcus* sp. 6301, manifestation of ultrastructure “fragility”, that is the absence (or transiency) of reversible changes, may be stated. This observation correlates with the known conservatism of both the metabolism of this cyanobacterium and its ultrastructural organization and cell morphology.

The most versatile forms of ultrastructural plasticity were characteristics of *C. fritschii*, possessing the most flexible metabolism and heterogeneity of the organization intracellular membrane structures and cell morphology among species under study.

The revealed forms of manifestation of ultrastructural plasticity of various species of cyanobacteria indicate the following possible adaptive mechanisms under unfavorable changes in the illumination regime: reversible thylakoid swelling, regulation of synthesis and degradation of glycogen, L-transformation, and population heterogeneity in terms of cell susceptibility to the same stimuli.

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