Chloroplasts

3

Shigeyuki Kawano

Chloroplasts are photosynthetic organelles found in plant cells and eukaryotic algae. They absorb sunlight and use it in conjunction with water and carbon dioxide gas to produce starch. Photosynthetic pigments such as chlorophylls capture the energy from sunlight, and RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) fixes carbon dioxide as a carbon source. Chloroplasts are not only the photosynthetic organelle but also the site of synthesis of many other important compounds such as pigments, fatty acids and amino acids. Chloroplasts, which are by definition plastids containing chlorophyll, and other plastids, such as etioplasts, leucoplasts, amyloplasts, and chromoplasts, develop either by division of an existing plastid or from proplastids. Proplastids arise during germ cell formation.

Chloroplasts are generally believed to have originated as endosymbiotic cyanobacteria. In this respect they are similar to mitochondria, but are found only in plants and photosynthetic protists. Both organelles are surrounded by a double membrane; both have their own DNA and are involved in energy metabolism. There are two main types of plastids depending on their membrane structure: primary plastids and secondary plastids. Primary plastids are found in glaucophytes, red, and green algae, including land plants, and secondary plastids are found in chlorophyll *c*-possessing algae, euglenophytes and chlorarachniophytes. Exploring the origin of plastids provide an insight into our understanding of the basis of photosynthesis in green plants, our primary food source.

In this chapter, various kinds of chloroplasts in land plants and algal cells will be highlighted, and related apparatuses will be illustrated. In the first three articles, S. Miyagishima et al., Y. Yoshida et al., and H. Hashimoto et al. review chloroplast division machinery in the unicellular red alga, *Cyanidioschyzon merolae* and the glaucocystophyte alga, *Cyanophora paradoxa*. Pyrenoids are sub-cellular compartments found in many algal chloroplasts, and their main function is to act as centers of carbon dioxide fixation in which ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is thought to be accumulated. T. Osafune et al. demonstrate the distribution of RuBisCO in synchronized *Euglena* cells by immunoelectron microscopy, showing RuBisCO accumulates in the pyrenoid. *Haematococcus pluvialis* is a freshwater species of green algae which is well known for its accumulation of carotenoids (e.g., astaxanthin) during encystment. S. Ota et al. present ultrastructural 3D reconstructions based on over 350 serial sections per cell to visualize the dynamics of astaxanthin accumulation and subcellular changes during encystment.

Many lower land plants (i.e., archegoniate plants) have cells containing only a single chloroplast. M. Shimamura et al. present monoplastidic cells of the hornwort, *Anthoceros punctatus*, and a liverwort, *Blasia pusilla*. Unique aquatic angiosperm *Podostemaceae* plants (riverweeds) have two differently sized chloroplasts in each epidermal cell. R. Fujinami presents dimorphic chloroplasts in the epidermis of *Podostemaceae* plants. A. Kondo et al. and C. Saito describe the distribution of chloroplasts and mitochondria in mesophyll cells of the flowering plant, *Kalanchoë blossfeldiana* and in *Sorghum* (a genus of numerous species of grasses). Y. Hayashi presents prolamellar bodies of the etioplast in etiolated cotyledon in the model land plant, *Arabidopsis thaliana*. Finally, H. Kuroiwa and T. Kuroiwa present the chloroplast division machinery of *Pelargonium zonale*, and Y. Nishimura presents active digestion of paternal chloroplast DNA in a young zygote of a model green alga, *Chlamydomonas reinhardtii*.

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Plate 3.1

Chloroplast division by the plastid-dividing ring

Plastids evolved from a cyanobacterial endosymbiont and their continuity is maintained by plastid division and segregation which is regulated by the eukaryotic host cell. Plastids divide by constriction of the inner and outer envelope membranes [1]. Ring-like structures called plastid-dividing rings have been identified in the red alga *Cyanidium caldarium* by transmission electron microscopy [2], and, since their discovery, plastid-dividing rings have been identified in several lineages of photosynthetic eukaryotes including land plants. Based on these observations, recent studies have identified several components of the plastid division machinery. The division complex has retained certain components of the cyanobacterial division complex along with components developed by the host cell [1]. Based on the molecular components of the division complex which have been identified, it is becoming increasingly clear how the division complex has evolved and how it is assembled, constricted, and regulated in the host cell.

These images show a dividing chloroplast of the unicellular red alga *Cyanidioschyzon merolae* as visualized by transmission electron microscope (**A**, TEM) and a field emission scanning electron microscope (**B**, FE-SEM). For TEM, cell and chloroplast division of *C. merolae* were synchronized by exposing cells to a light/dark cycle. Synchronized cells were rapidly frozen in liquid propane (−195 ºC) and fixed with 1 % OsO_4, then dissolved in acetone at −80 ºC. After samples were warmed gradually to room temperature, they were embedded in Spurr resin. Serial thin sections (each 70 nm thick) were stained with uranyl acetate and lead citrate, and examined with TEM [3]. For FE-SEM, dividing chloroplasts were isolated from synchronized *C. merolae* culture and were fixed with 1 % glutaraldehyde. After dehydration, chloroplasts were dried to the critical point, and then mounted and sputter-coated with platinum. The samples were examined with FE-SEM [4]. Scale bars: 0.5 μm. This figure is adapted from [4].

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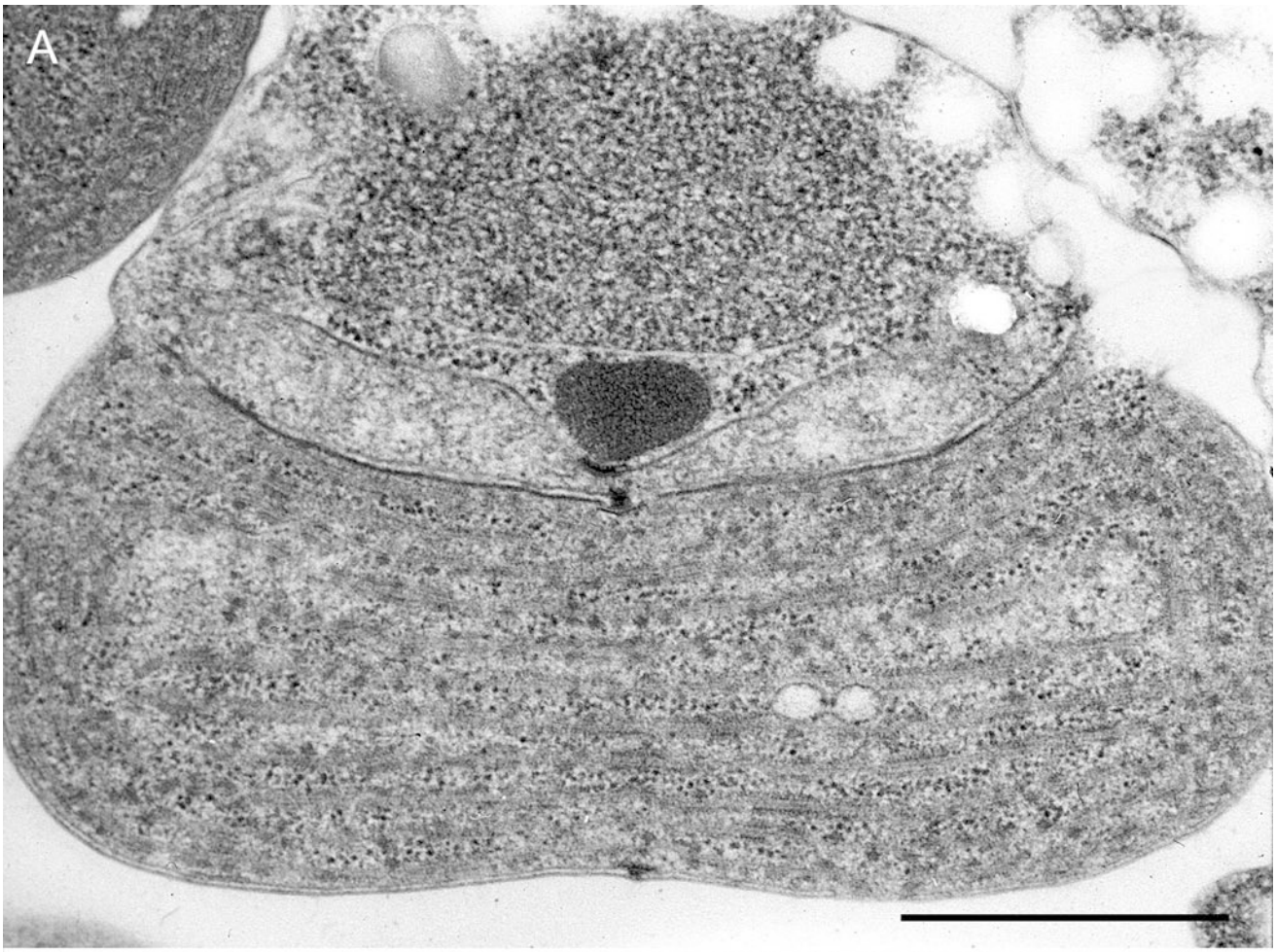
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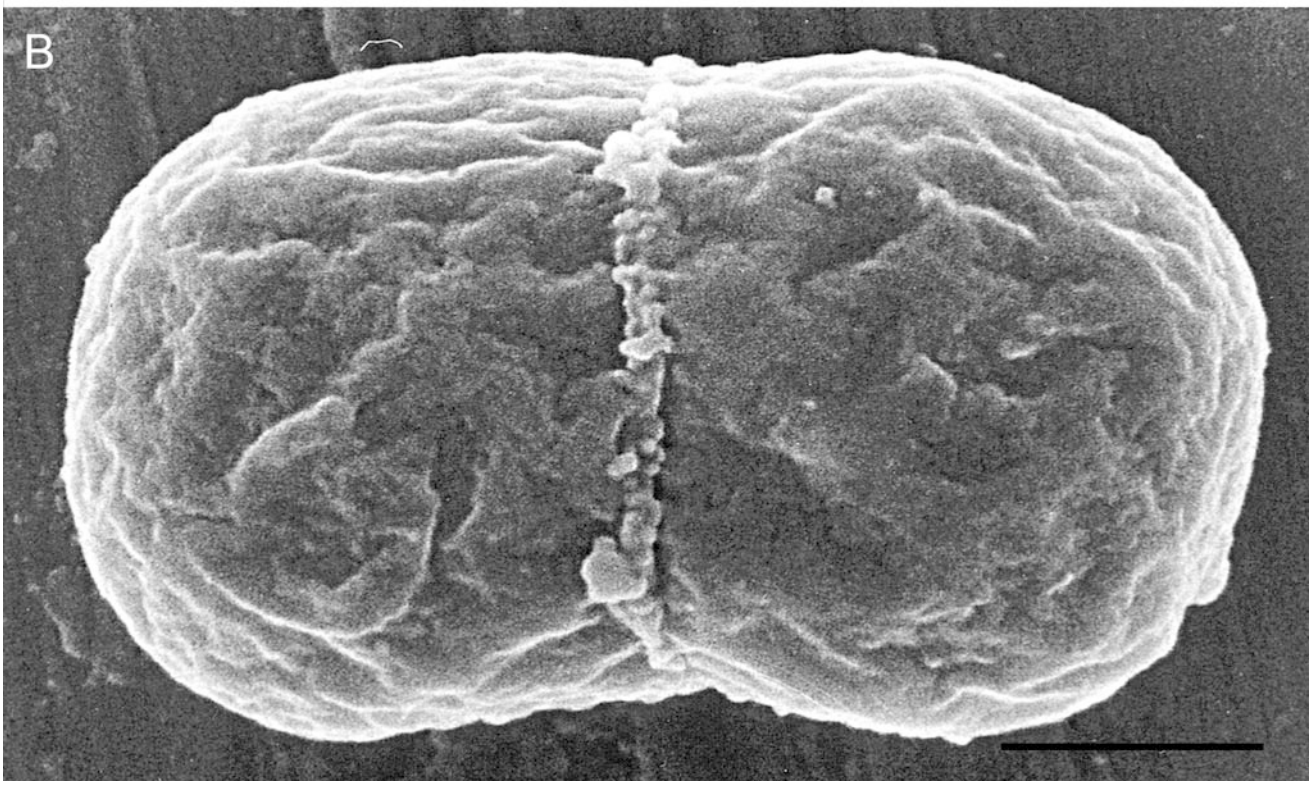
B

Plate 3.2

Chloroplasts divide by contraction of a bundle of polyglucan nanofilaments

Plastids such as chloroplasts arose from a cyanobacterial endosymiont and have retained their own genome. Consistent with their bacterial origin, plastids multiply by binary division of pre-existing organelles, which is executed by a complex called the plastid dividing (PD) machinery [5]. The glycosyltransferase protein plastid-dividing ring 1 (PDR1) was identified by proteomic analysis of PD machinery isolated from unicellular *Cyanidioschyzon merolae*, which contains a single chloroplast and a single mitochondrion [5]. Together with carbohydrates the PDR1 protein forms a ring that constricts to physically divide the plastid. Figures of A and B show an immuno-electron (EM) micrograph (**A**) and an immunofluorescence image (**B**) of isolated PD machinery. Immunogold particles indicating PDR1 (black dots) are localized throughout each of the PD machineries. Many more immunogold particles appear in the less condensed PD ring filament region than in the solid PD ring filament region, suggesting that PDR1 proteins are associated with the whole of the PD ring, from the inside to the outside. Fluorometric saccharide ingredient analysis of purified PD ring filaments showed that only glucose was included. Thus, the PD ring is made up of polysaccharide chains and proteins, which together generate a ring that constricts to divide the plastids. The EM image was chosen as the cover of *Science* (Vol. 329, no. 5994). Recently, it was revealed that mitochondrial and plastid divisions are regulated by a kinesin-like protein TOP (green) (**C**) [6]. In the early phase of division, TOP promotes Aurora kinase localization to activate division machineries by protein phosphorylation. A series of studies have uncovered important and unexpected cooperative behaviors of organelle division machineries and cell proliferation mechanisms.

In order to isolate intact PD machineries, dividing plastids were isolated from synchronized *C. merolae* cells at M phase. Isolated PD machineries form not only ring but also spiral and supertwist structures (**D**) [7]. The immunofluorescence image (**D**) was chosen as the highlight of *Science* (Vol. 313, no. 5792). In figure B, an isolated PD machinery was immunostained with PDR1 (green), Dnm2 (red) and FtsZ2 (blue). For immuno-EM, samples were negatively stained with 0.5 % phosphotungstic acid (pH 7.0) and examined with an electron microscope. Scale bars: 0.1 μm (**A**), 1 μm (**B**, **C**). This figure is adapted from [5, 6, 7].

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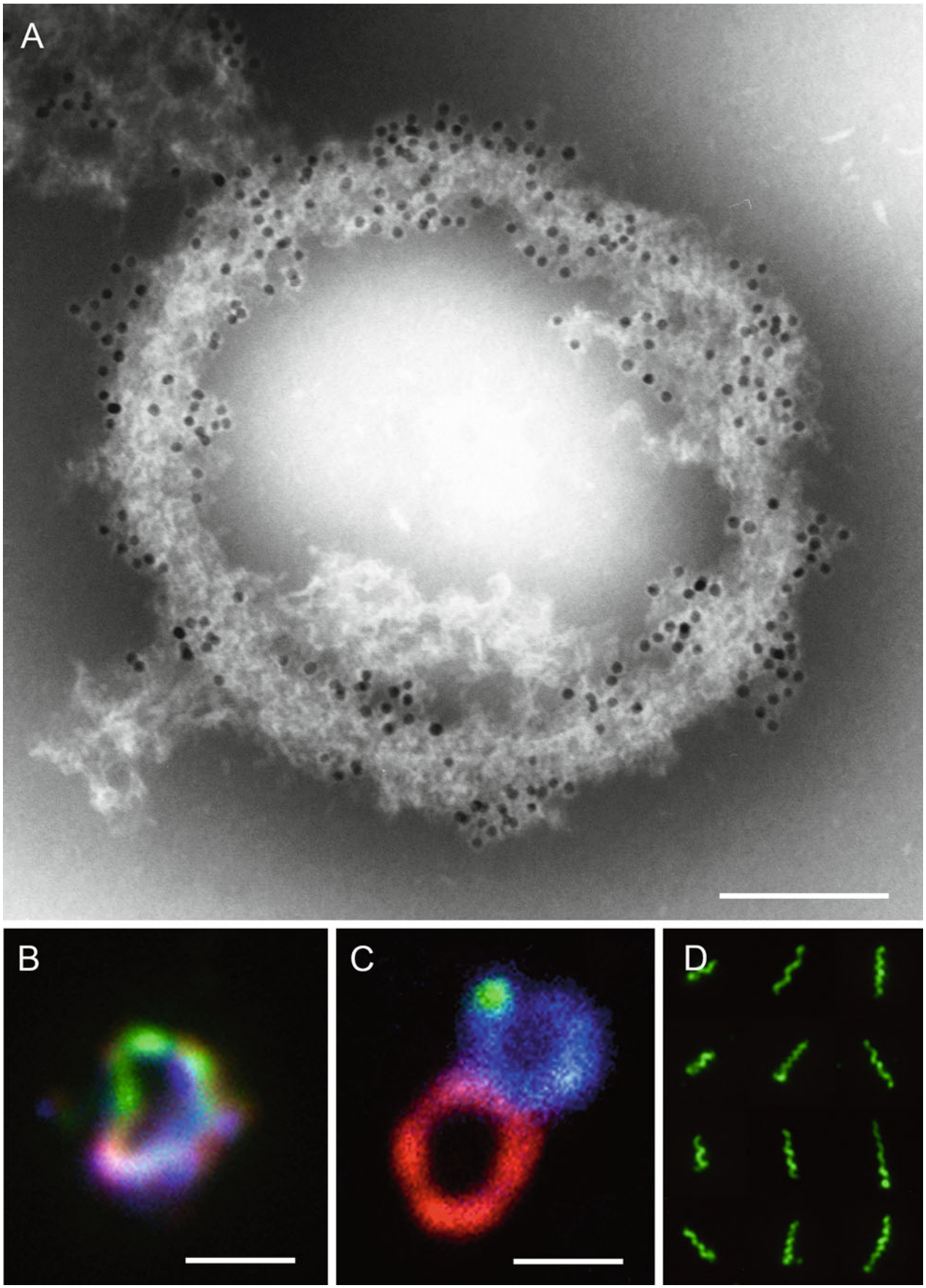
A
B
C
D

Plate 3.3

Cyanelle division of the glaucocystophyte alga *Cyanophora paradoxa*

Glaucocystophyte plastids are called cyanelles because they are surrounded by a peptidoglycan layer, resembling cyanobacterial cells, which are the putative ancestor of plastids. This suggests that cyanelles may be the most primitive among the known plastids, and this idea is supported by the sequence phylogeny of cyanelle DNA.

Cyanelles divide by binary fission as do other plastids. However, electron microscopic observations reveal that cyanelle division differs from typical plastid fission. Cyanelle division involves septum formation at the cleavage site by ingrowth of the peptidoglycan layer between the outer and inner envelope membranes. Electron-opaque rings are detected only on the stromal face of the inner envelope membrane (**C**, white arrow) but not on the cytoplasmic face of the outer envelope membrane (**C**) [8]. SEM images also confirm the absence of a cytoplasmic ring (**A**) [9]. As in cyanobacterial cell division, septum ingrowth may in part provide the external fission force, whereas in plastids without peptidoglycan the fission force may be provided by the cytoplasmic plastid-division ring. On the other hand, fluorescence immunostaining shows evidence for the presence of a FtsZ ring at the cleavage site (**B**, green fluorescence; white and red represent fluorescence of DAPI-stained nucleoids and chlorophyll autofluorescence, respectively) [10], a homologue of the bacterial cell division ring which is commonly present in plastids of other plants. These observations suggest that cyanelle division represents an intermediate stage between cyanobacterial and plastid division, a potential "missing link." If plastids have a monophyletic origin, the stromal ring, i.e. cyanelle ring, and homologous inner plastid-dividing ring in other plastids might have evolved before than the outer cytoplasmic plastid-dividing ring.

Cyanophora paradoxa Korshikov was obtained from strain NIES-547 of the algal collection of the National Institute of Environmental Studies (Tsukuba, Japan). Cells were axenically grown in C-medium at 25 °C under a diurnal 13:11 h light:dark regime. Cultures were illuminated with white fluorescent lamps at an intensity of approximately 50 μmol photons m-2 s-1. *C. paradoxa* cells were harvested by centrifugation at 1,000 g at room temperature and then fixed with 2.5 % (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) on ice for 2 h. They were washed with the same buffer several times and post-fixed with 1 % (w/v) osmium tetroxide in the same buffer on ice for 2 h. Thereafter, cells were rinsed with the same buffer several times and dehydrated through a graded acetone series on ice. Samples were then infiltrated and embedded in Spurr resin. Ultrathin sections with grey–silver interference color were stained with uranyl acetate and lead citrate, then observed under a transmission electron microscope. Scale bars: 0.2 μm. This figure is adapted from [9, 10].

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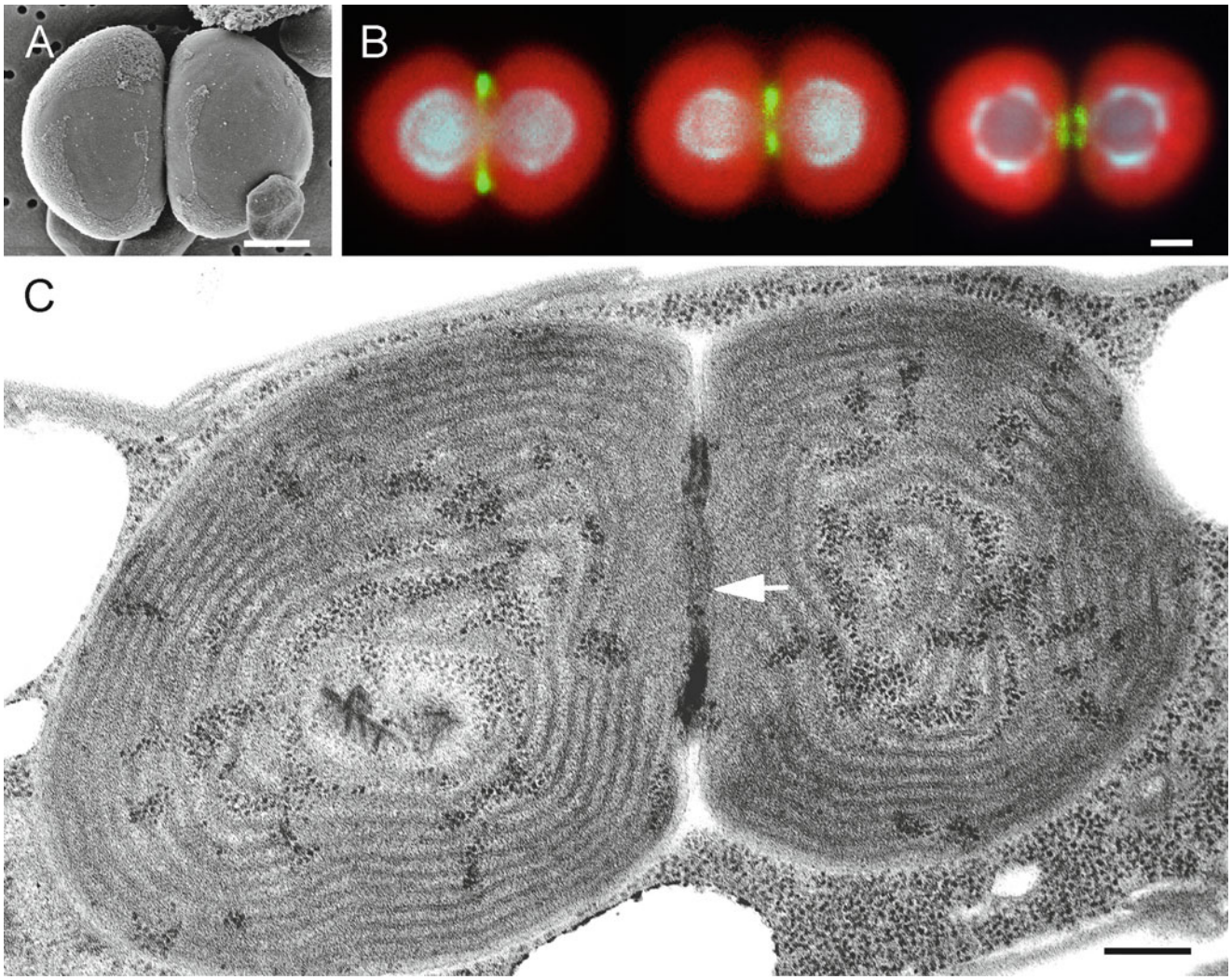
A
B
C

Plate 3.4

3D distribution of RuBisCO in synchronized *Euglena* cells

The pyrenoid exists in the chloroplasts of most eukaryotic algae and moss plants, and has been thought a mere storage place of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), though its actual functions are poorly understood.

Euglena gracilis cells were synchronized under a 14 h light and 10 h dark regimen at 25 °C under photoautotrophic conditions. Cell grew during the light period and divided during the following 10 h period, whether cells were in the dark or in the light [11]. Changes in pyrenoid morphology and the distribution of RuBisCO in the *Euglena* chloroplast were observed by immunoelectron microscopy [12] at different phases of the cell cycle in synchronized culture under photoautotrophic conditions. Immunoreactive proteins were concentrated in the pyrenoid (**A**) [13], and less densely distributed in the stroma during the light period, and gold particles are localized in the pyrenoid during the light period, which disappeared during the dark period with RuBisCO dispersing throughout the stroma [11]. Toward the end of the division phase, the pyrenoid began to form in the center of the stroma, and RuBisCO is again concentrated in the pyrenoid. A 3D reconstruction of the distribution of RuBisCO (**B**, **left**) and a 3D reconstruction of the main pyrenoid, satellite pyrenoids, and thylakoid membranes super-imposed upon the 3D distribution of RuBisCO (**B**, **right**) were generated. It was found that approximately 80 % of total RuBisCO was localized to the pyrenoid region (**B**) [13]. Time courses of changes in photosynthetic CO_2-fixation and the carboxylase activity of RuBisCO in *Euglena* cells during the cell cycle in synchronized cultures are shown. From a comparison of photosynthetic CO_2-fixation with the total carboxylase activity of RuBisCO extracted from *Euglena* cells in the growth phase, it is suggested that carboxylase in the pyrenoid functions in CO_2-fixation during photosynthesis [11]. Gold dots: RuBisCO proteins. Red shows the main and smaller satellite pyrenoids. Thylakoids (green). Scale bars: 5 μm. This figure is adapted from [11].

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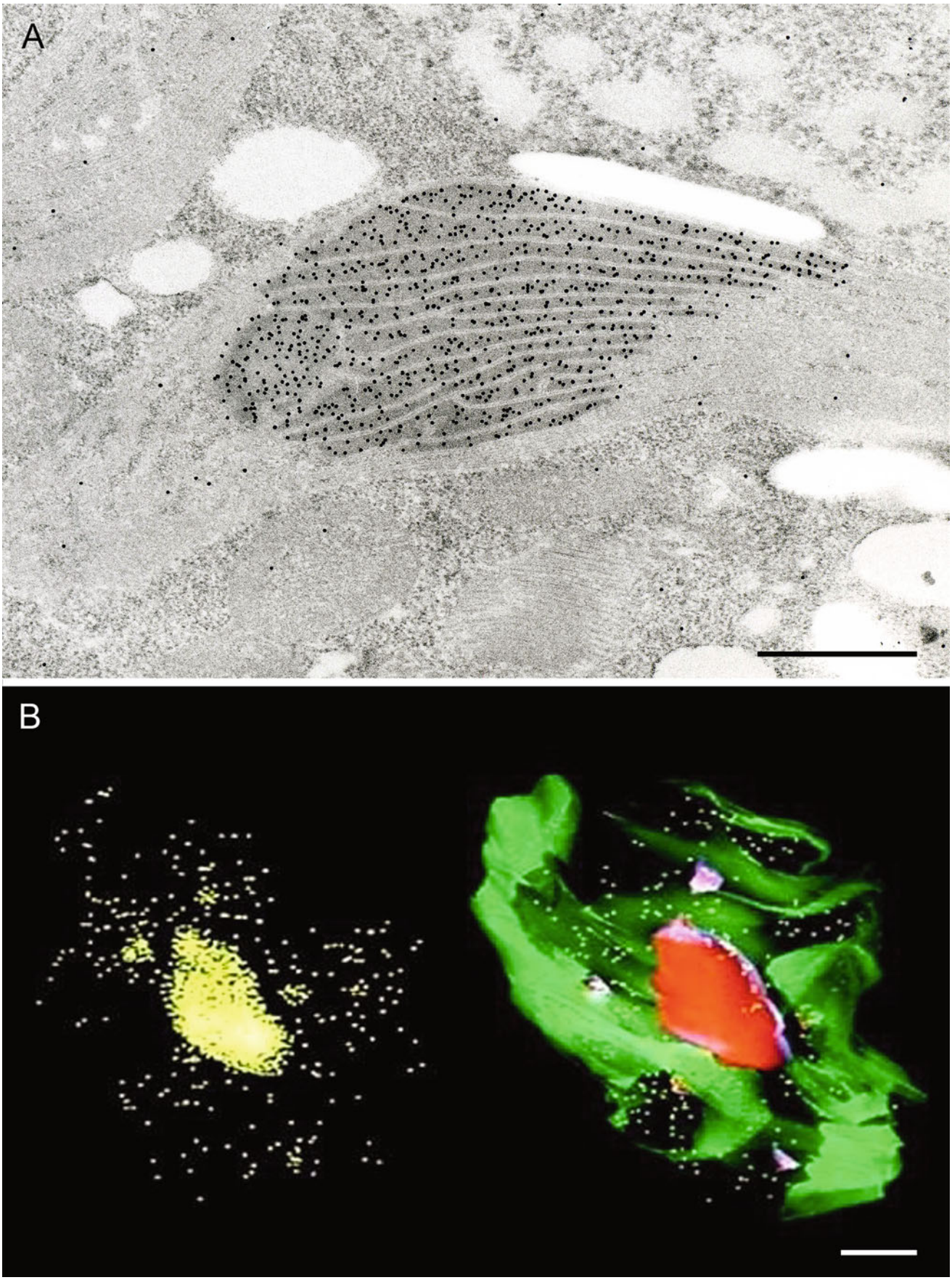
A
B

Plate 3.5

Developing and degenerating chloroplasts in *Haematococcus pluvialis*

Haematococcus pluvialis is a freshwater green algal species and is well known for its accumulation of the strong antioxidant astaxanthin, which is used in aquaculture, various pharmaceuticals, and cosmetics. High levels of astaxanthin are accumulated in cysts. It is not understood, however, how high levels of astaxanthin, which is soluble in oil, accumulate during possible during encystment. Ultrastructural 3D reconstruction was performed based on over 350 serial sections per cell to visualize the dynamics of astaxanthin accumulation and subcellular changes during the encystment of *H. pluvialis*. This study showcases marked dynamics of subcellular elements, such as chloroplast degeneration, in the transition from green coccoid cells to red cyst cells during encystment. In green coccoid cells, chloroplasts account for 41.7 % of the total cell volume and the relative volume of astaxanthin was very low (0.2 %). In contrast, oil droplets containing astaxanthin predominate in cyst cells (52.2 %), in which the total chloroplast volume is markedly decreased (9.7 %). Volumetric measurements also demonstrate that the relative volumes of the cell wall, starch grains, pyrenoids, mitochondria, Golgi apparatus, and the nucleus are smaller in a cyst cell than in green coccid cells. This indicates that chloroplasts are degraded, resulting in a net-like morphology, but chloroplasts do not completely disappear in the red cyst stage [14].

This figure shows 3D images of a green coccid cell (**A**) and a red cyst cell (**B**). Cells of *H. pluvialis* were fixed with 2.5 % glutaraldehyde and 2.5 % $KMnO_4$ (green coccid cells) or 2.5 % glutaraldehyde and 1 % OsO_4 (red cyst cells). Ultrathin serial-sections were cut on a ultramicrotome using a diamond knife, and images were obtained using a transmission electron microscope (TEM) at 100 kV. Digital TEM images were trimmed using Adobe Photoshop and printed on A4 paper sheets. Contours of each subcellular element were traced manually using color marker pens, and then the images were scanned and converted into digital images (JPG format). 3D images were subsequently reconstructed using software. Scale bar: 5 μm. This figure is adapted from [14].

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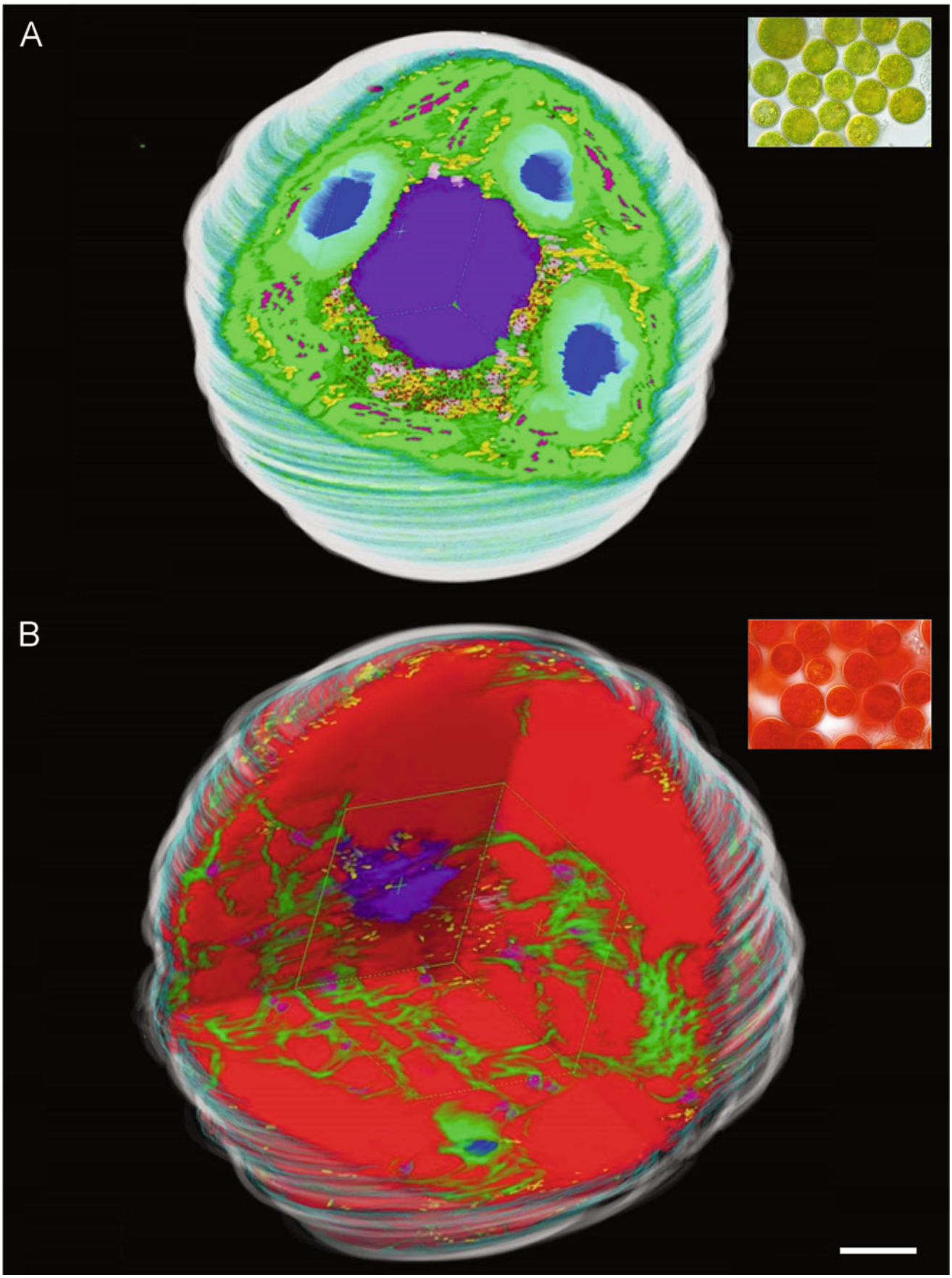
A
B

Plate 3.6

Monoplastidic cells in lower land plants

Although the cells of seed plants contain a few dozen to hundreds of plastids, many lower land plants (archegoniate plants) have cells that contain only a single plastid [15, 16]. The cells in vegetative dividing tissues of hornworts and some microphyllophytes (*Isoetes* and *Selaginella*) are typically monoplastidic (contain only one or two chloroplasts). In reproductive cell lineages, monoplastidic conditions are more commonly observed. For example, sporocytes (spore mother cells) of bryophytes (all mosses, hornworts, and some liverworts), microphyllophytes and some ferns (*Isoetes*, *Selaginella*, *Lycopodium* and *Angiopteris*) are typically monoplastidic. Additionally, sperm cells of all bryophytes and some microphyllophytes are monoplastidic. Since DNA containing organelles (nucleus, mitochondria and plastid) do not arise de novo in the cell, they should be transmitted to each daughter cell through cell division. In monoplastidic cells, morphogenetic plastid division seems to ensure the allotment of a plastid to the daughter cells. The single plastid divides before nuclear division and divided plastids serve as microtubule organizing centers for mitotic apparatuses such as the centrosome [17] (see Plate 6.5). The monoplastidic state of green algae and many archegoniate plants is believed to be an ancestral character. During the evolution of land plants, establishment of the polyplastidic condition might have allowed a more effective plastid distribution for photosynthesis.

The upper figure (**A**) shows monoplastidic cells in the gametophytic vegetative tissue of a hornwort, *Anthoceros punctatus*. Each chloroplast contains a pyrenoid similar to that of green algae (see Plate 3.4). The lower figure (**B**) shows a sporocyte of a liverwort, *Blasia pusilla*. In meiotic prophase, the single chloroplast divides twice in advance of meiotic nuclear division and the four resultant chloroplasts migrate to equidistant positions in the sporocyte. The sporocyte cytoplasm then lobes into four future spore domains associated with chloroplast division and migration. Scale bars: 10 μm.

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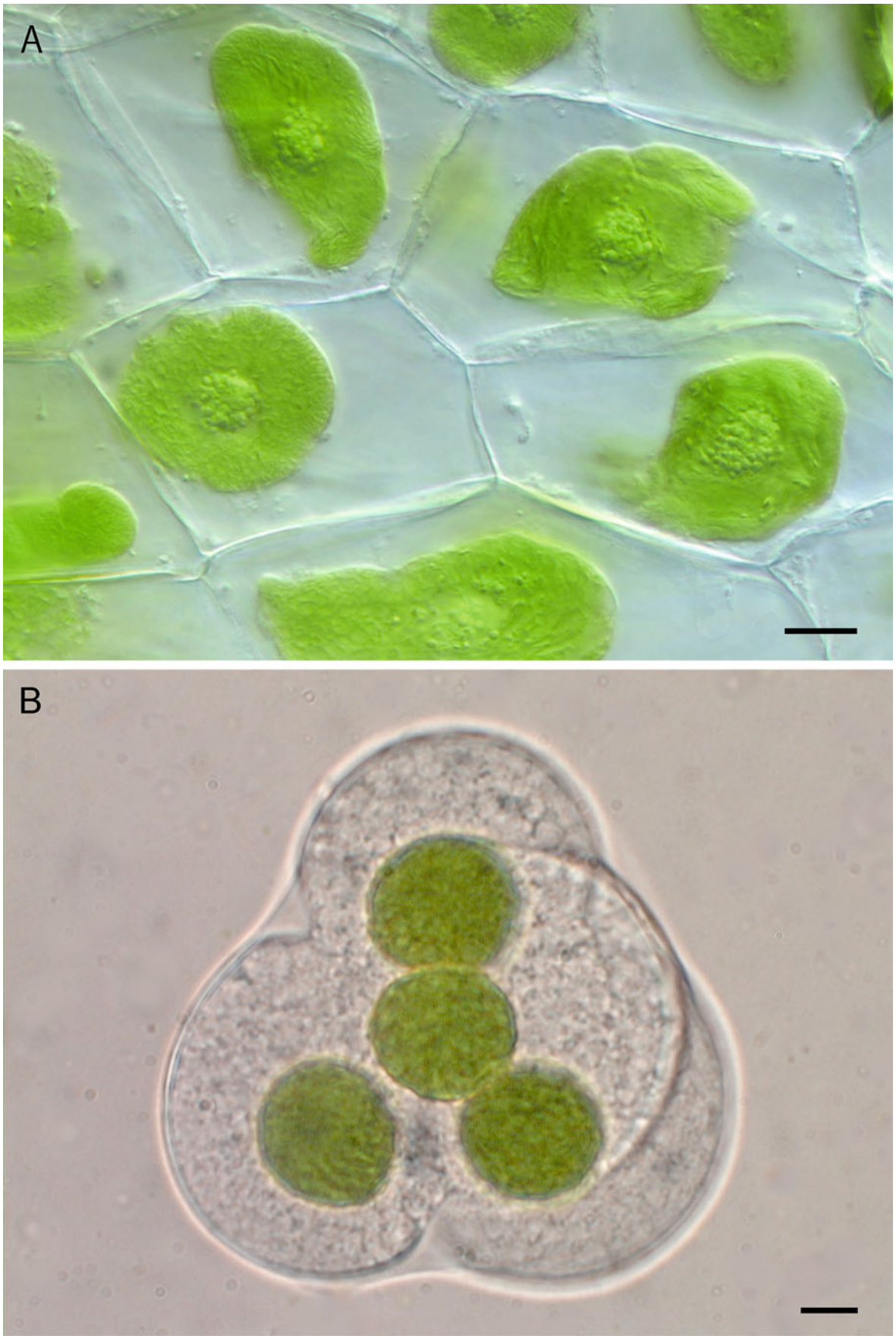
A
B

Plate 3.7

Dimorphic chloroplasts in the epidermis of the aquatic angiosperm *Podostemaceae* family

Plants of the *Podostemoideae*, a subfamily of the unique aquatic angiosperm family *Podostemaceae*, which are found in rapids and waterfalls of the tropics and subtropics, have two different sized chloroplasts in each epidermal cell [18]. Such dimorphic chloroplasts have not been reported in any other angiosperm, suggesting that the dimorphism represents an adaptation to the unique habitat of the *Podostemaceae*. Plant bodies in this family are submerged in water and subjected to torrential water during the rainy season, and emerge above the water surface to produce flowers and fruits in the dry season.

This figure shows the small and the large chloroplasts in the root epidermal cells of *Hydrobryum khaoyaiense*. Small and large chloroplasts are located separately along the upper and lower tangential walls of each epidermal cell, respectively, with no chloroplasts along the radial walls (**A**). Small chloroplasts are approximately one-sixth the size of large chloroplasts, significantly smaller in area (3.7 ± 1.8 μm^2, $N = 70$ chloroplasts from 15 cells) than the large ones (23.7 ± 11.0 μm^2, $N = 37$ from 15 cells) (Student's t-test, $p < 0.01$) [18]. Magnified images of one small chloroplast and one large chloroplast are shown in **B** and **C**, respectively. Ultrastructurally, small and large chloroplasts both contain normal grana and osmiophilic granules. Large chloroplasts haver four to five (and sometimes up to eight) thylakoid layers per granum, and well developed large starch grains (**C**). Large chloroplasts are identical to chloroplasts in mesophyll and parenchyma cells in their size and ultrastructure. On the other hand, small chloroplasts contained three to four thylakoid layers per granum, but have very few starch grains (**B**), and hence, the small chloroplasts may perform unique functions. *Podostemaceae* utilize HCO_3^- as a source of CO_2 for photosynthesis like the majority of submerged freshwater angiosperms. Therefore, it may be possible that the small chloroplasts function mainly to supply energy to CO_2 uptake process via HCO_3^- pump.

Roots of *Hydrobryum khaoyaiense* M. Kato were fixed in the field with 1.6 % glutaraldehyde (GA) in river water (Haew Narok Waterfall, Khao Yai National Park, Thailand) to avoid chloroplast movement in response to changing light directions. All fixed materials were kept at 4 °C for 24 h, then post-fixed in 1.0 % osmium tetroxide in 0.05 M phosphate buffer (pH 7.2) for 1 h at 4 °C. Samples were dehydrated in an ethanol series and embedded in epoxy resin (Plain Resin; Nissin EM). For TEM, ultrathin sections (70 nm thick) were stained with uranyl acetate and lead citrate and imaged using a transmission electron microscope. Ch^{-L}, large chloroplast;Ch^{-S}, small chloroplast. Scale bars: 5 μm (**A**), 1 μm (**B**, **C**).

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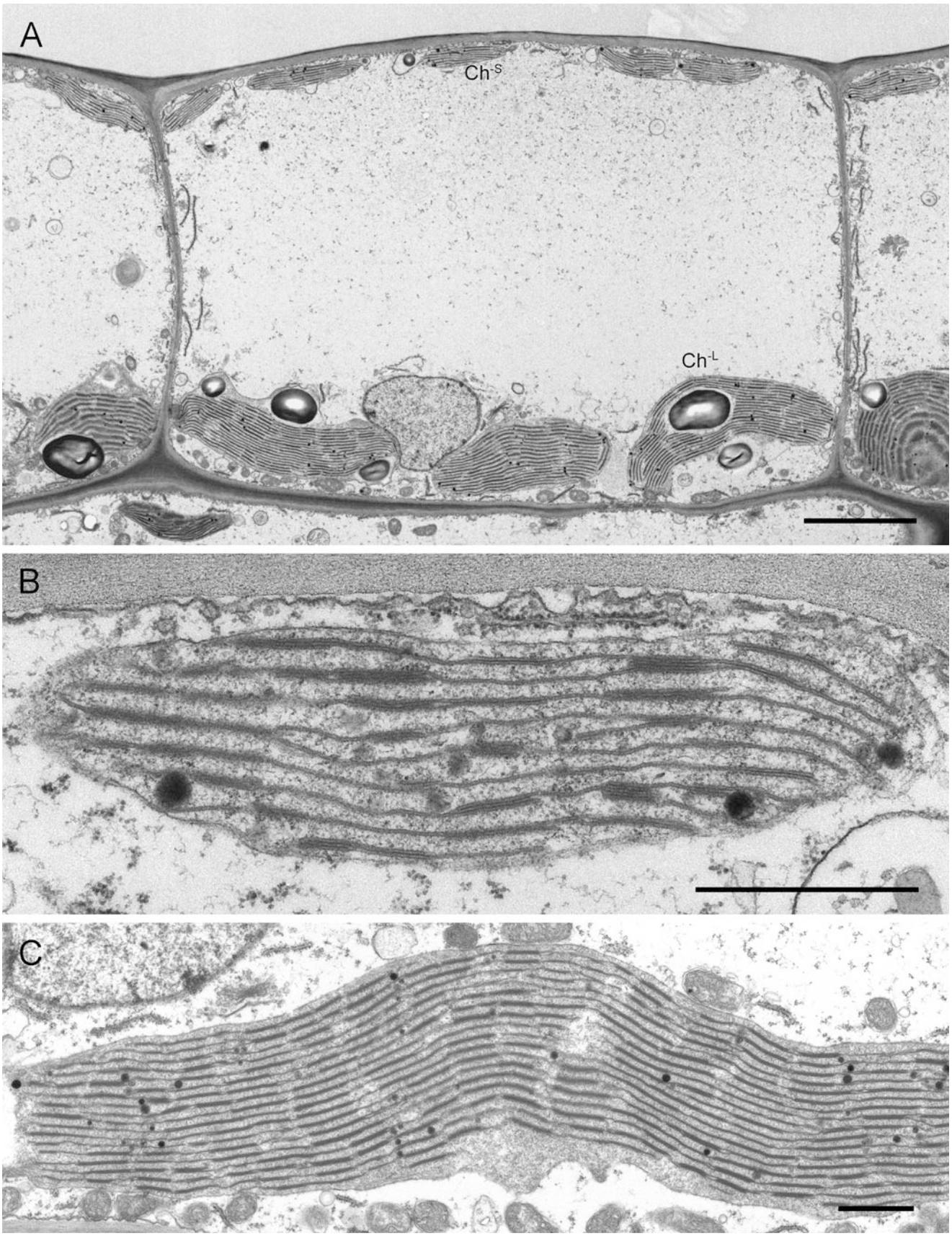
A
Ch-S
Ch-L
B
C

Plate 3.8

Distribution of chloroplasts and mitochondria in *Kalanchoë blossfeldiana* mesophyll cells

In leaves of some succulent crassulacean-acid-metabolism (CAM) plants, including species of the *Kalanchoë* genus, it was found that chloroplasts clumping is induced by a combination of light and water stress. In leaves of plants withheld from water for 10 days (water-stressed plant), chloroplasts clump densely in under light conditions and disperse during darkness. Chloroplast clumping results in leaf optical changes, namely a decrease in absorbance and an increase in transmittance. The plant stress hormone abscisic acid induces chloroplast clumping in leaf cells under light conditions, suggesting that this phenomenon in succulent plants is a morphological mechanism that protects against light stress intensified by a severe water deficiency [19].

Shown here are confocal microscopic images showing the distribution of chloroplasts (red, autofluorescence) and mitochondria (green, Rhodamine 123) in mesophyll cells of well-watered *Kalanchoë blossfeldiana* plants (**A**) and water-stressed plants (**B**) [20]. The inset figure is an enlargement of the rectangle in (**A**). In the leaves of well-watered plants, chloroplasts and mitochondria in mesophyll cells are dispersed across a wide area (**A**), and some mitochondria are located adjacent to each chloroplast (**inset** in **A**). In the leaves of water-stressed plants exposed to light, chloroplasts become densely clumped in mesophyll cells (**B**). The intracellular locations of the chloroplast clumps varied. Although many mitochondria were observed in the chloroplast clump, they were also distributed to other areas of the mesophyll cells (**B**). There were a few mitochondria around each chloroplast under both well-watered and water-stressed conditions. The positioning of mitochondria adjacent to chloroplasts seems to be essential for chloroplast functioning [20].

Leaf segments were hand-sectioned with a razor blade and stained with fluorescent agents under dark conditions. For mitochondria, sections were incubated in 1 μg/mL Rhodamine 123 solution for 2 m; leaf segments were not fixed because Rhodamine 123 cannot stain mitochondria under such conditions. Stained sections were observed under a confocal laser microscope. Clumped chloroplasts, cCH; mesophyll cell, Me; chloroplast, arrow; mitochondria, arrowhead. Scale bars: 50 μm (**main**), 10 μm (**inset**). This figure is adapted from [20].

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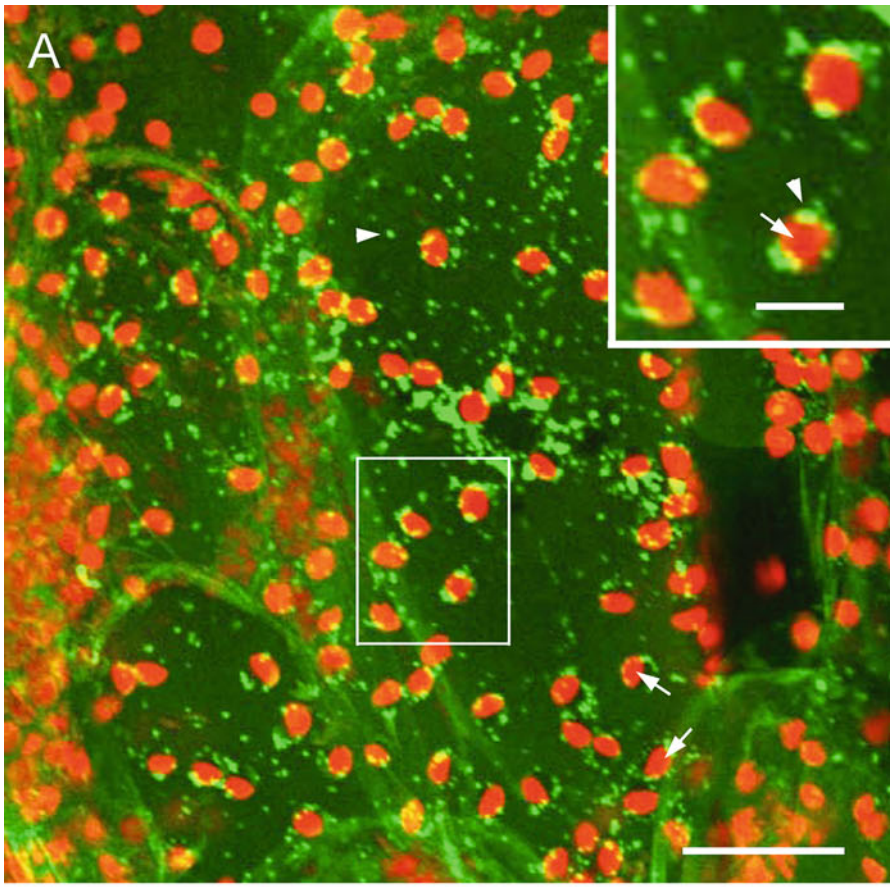
A

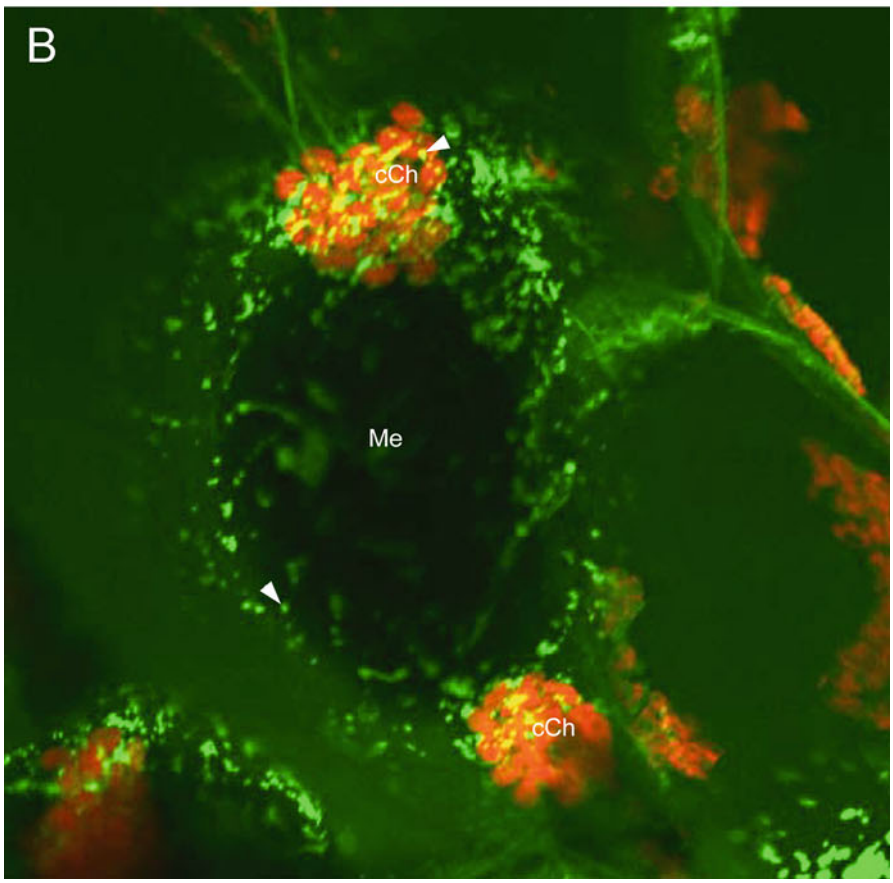
B
cCh
Me
cCh

Plate 3.9

Etioplast prolamellar bodies in *Arabidopsis thaliana* etiolated cotyledon

When plants are grown in the dark cotyledons are white or yellow and plastids undergo limited development in etiolated cotyledon. Etioplasts are present in the cells of etiolated cotyledon. Upon introduction to light, normal growth resumes and the etiolated cotyledon turns green and begins to photosynthesize. The etioplast is a special state in transitioning from the proplastid to a normal, fully functioning chloroplast. Etioplasts are not an intermediate in normal chloroplast development. Proplastids become chloroplasts in plants grown in the light, or become etioplasts in plants kept in darkness. The characteristic proteins of mature chloroplasts are absent or present in very low amounts in etioplasts, although they contain some of the lipids and a precursor pigment called protochlorophyllide, but no chlorophyll [21]. The continued synthesis of lipids without synthesis of thylakoid protein leads to the structure of the prolamellar body which consists of tubes that branch in three dimensions. The prolamellar bodies form a quasi-crystalline lattice whose continuous surface is curved in opposite directions with a continuous compartment inside the tubes. The tetrahedral membrane lattice is the most common arrangement featuring branched, tubular repeating units interconnected in three dimensions.

In order to detect the membranes of prolamellar bodies, endplasmic reticulums and Golgi apparatus in the cell we performed the following fixation. Etiolated cotyledons were collected from plants grown on growth medium for 5 days in the dark at 22 °C. Samples were fixed for 3 h at 4 °C in cacodylate buffer (pH 7.4) containing 4 % paraformaldehyde, 1 % glutaraldehyde, and 0.1 M $CaCl_2$ washed with 0.1 M cacodylate buffer for 1.5 h, postfixed with 2 % OsO_4 plus 0.8 % $K_3Fe(CN)_6$ and 1 μM $CaCl_2$ in 0.1 M cacodylate buffer for 2 h at room temperature, dehydrated serially in ethanol, embedded in Spurr resin, ultrathin-sectioned, stained with uranium and lead, and observed with an electron microscope [22]. Scale bar: 500 μm.

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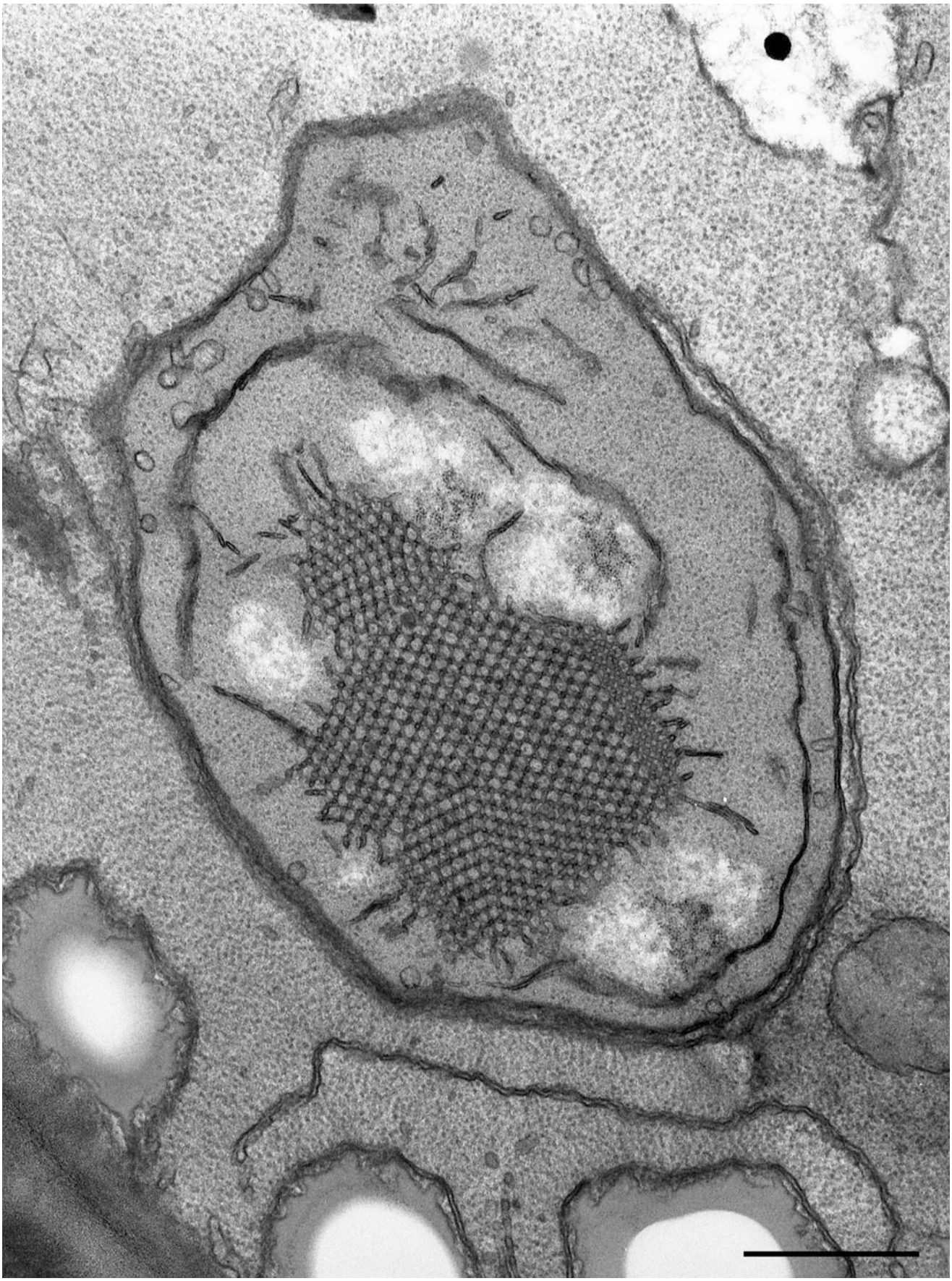

Plate 3.10

Chloroplasts and mitochondria in *Sorghum* bundle sheath cells

The vascular bundle of Sorghum leaf was observed by fluorescent microscopy (**A**) and transmission electron microscopy (**B**). Distinct structural differences between chloroplasts in mesophyll cells and bundle sheath cells are starkly apparent.

Before fixation, Sorghum leaf was cross-sectioned (<0.2 mm in thickness) by razor blade, and then cut into small pieces (<2 mm in length). Samples were then prepared for both transmission electron microscopy and fluorescent microscopy. For transmission electron microscopy, samples were rapidly frozen by high-pressure freezing. Frozen samples were then transferred to 4 % OsO_4 in anhydrous acetone and kept at −80 °C for 4 days. Samples were held at −20 °C for 2 h, 4 °C for 2 h, then at room temperature for 10 min, and washed with anhydrous acetone. Samples were then embedded in Spurr resin. Ultrathin sections were cut, stained, and observed under a transmission electron microscope. For fluorescent microscopy, samples were fixed with 2 % glutaraldehyde, dehydrated by ethanol washes, and embedded in Technovit 7100 resin. 1 μm sections were cut, stained with DAPI, and observed by fluorescent microscope. Scale bars: 10 μm (**A**), 5 μm (**B**).

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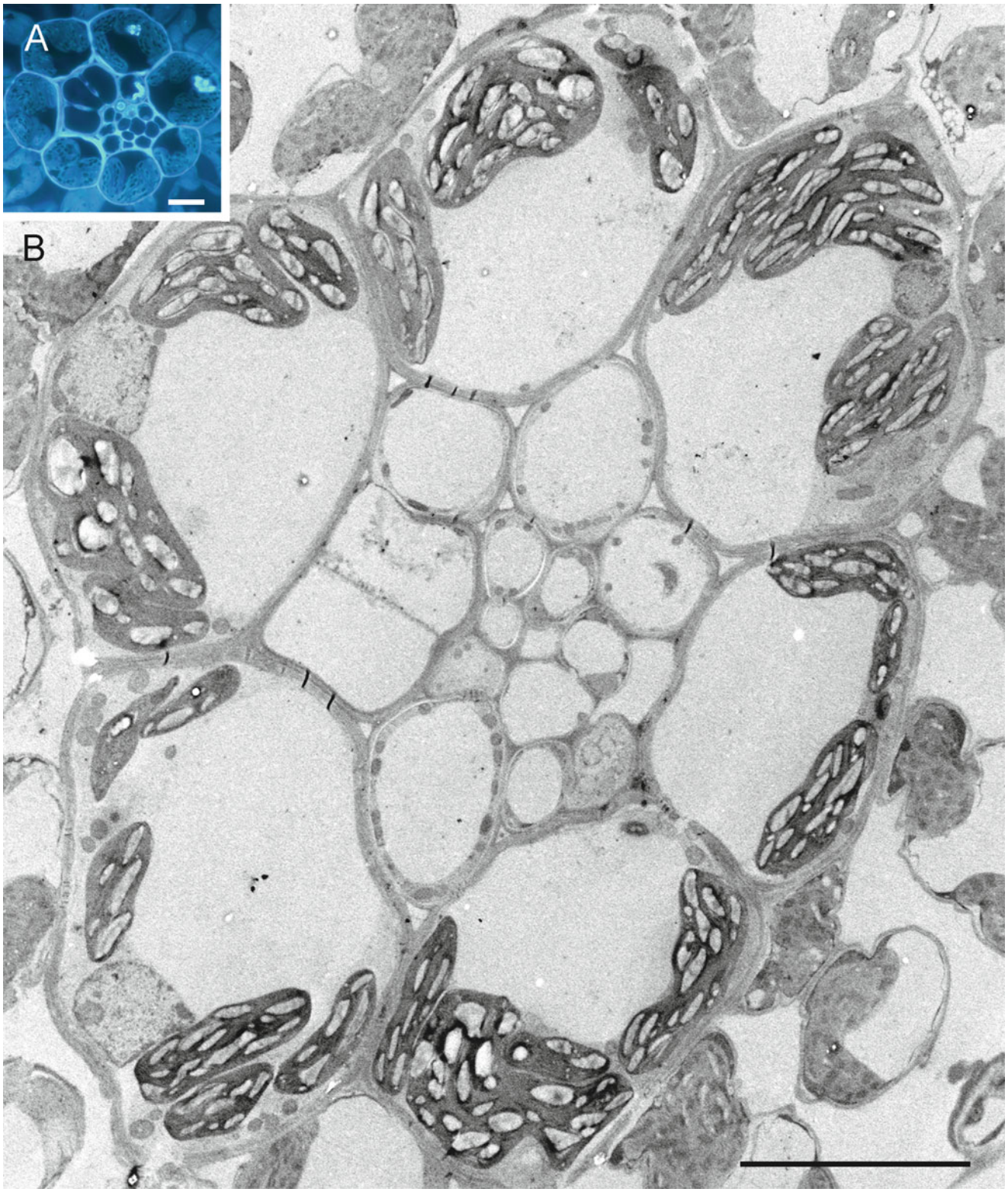
A
B

Plate 3.11

Chloroplast division machinery in *Pelargonium zonale*

Chloroplasts (plastids) ordinarily increase by binary fission, and electron-dense plastid-dividing rings (PD rings) form at the chloroplast division site. PD rings consist of inner and outer double (or triple) rings which physically divide the chloroplast. Chloroplasts are assumed to have arisen from bacterial endosymbionts, and bacterial division is instigated by the bacterial cytokinesis Z-ring protein (FtsZ). FtsZ genes have been identified in many algae and higher plants, and FtsZ localizes to co-aligned rings in chloroplasts. Immunofluorescence and electron microscopic evidence of chloroplast division via complex machinery involving the FtsZ and PD rings have been observed in the higher plant *Pelargonium zonale* Ait [23]. Prior to invagination of the chloroplast, the FtsZ protein attaches to a ring at the stromal division site. Following formation of the FtsZ ring, the inner stromal and outer cytosolic PD rings appear at the chloroplast constriction site during chloroplast division. Neither the FtsZ nor the inner PD rings change width, but the volume of the outer PD ring gradually increases. Based on these results, it appears that the FtsZ ring determines the division region, after which the inner and outer PD rings form as a lining for the FtsZ ring. With the outer ring providing the initial force, the FtsZ and inner PD rings ultimately decompose to their base components. These figures show chloroplast divisions in early embryos of *P. zonale*. Immunofluorescence images of chloroplasts labeled with anti-LlFtsZ antibody [24] are arranged from top to bottom according to chloroplast division state (chloroplasts emit red auto-fluorescence) (**A**). FtsZ (yellow-green fluorescence) was attached to rings at the division sites. Closed circular rings visualized as well as in **A** (**B**). Enlarged immunofluorescence image of dividing chloroplasts reacted with anti-LlFtsZ antibody (**C**). Electron microscopic image showing PD ring of dividing chloroplast (**D**). Insets show the chloroplast dividing site with inner and outer PD rings. Scale bars: 1 μm. This figure is adapted from [23].

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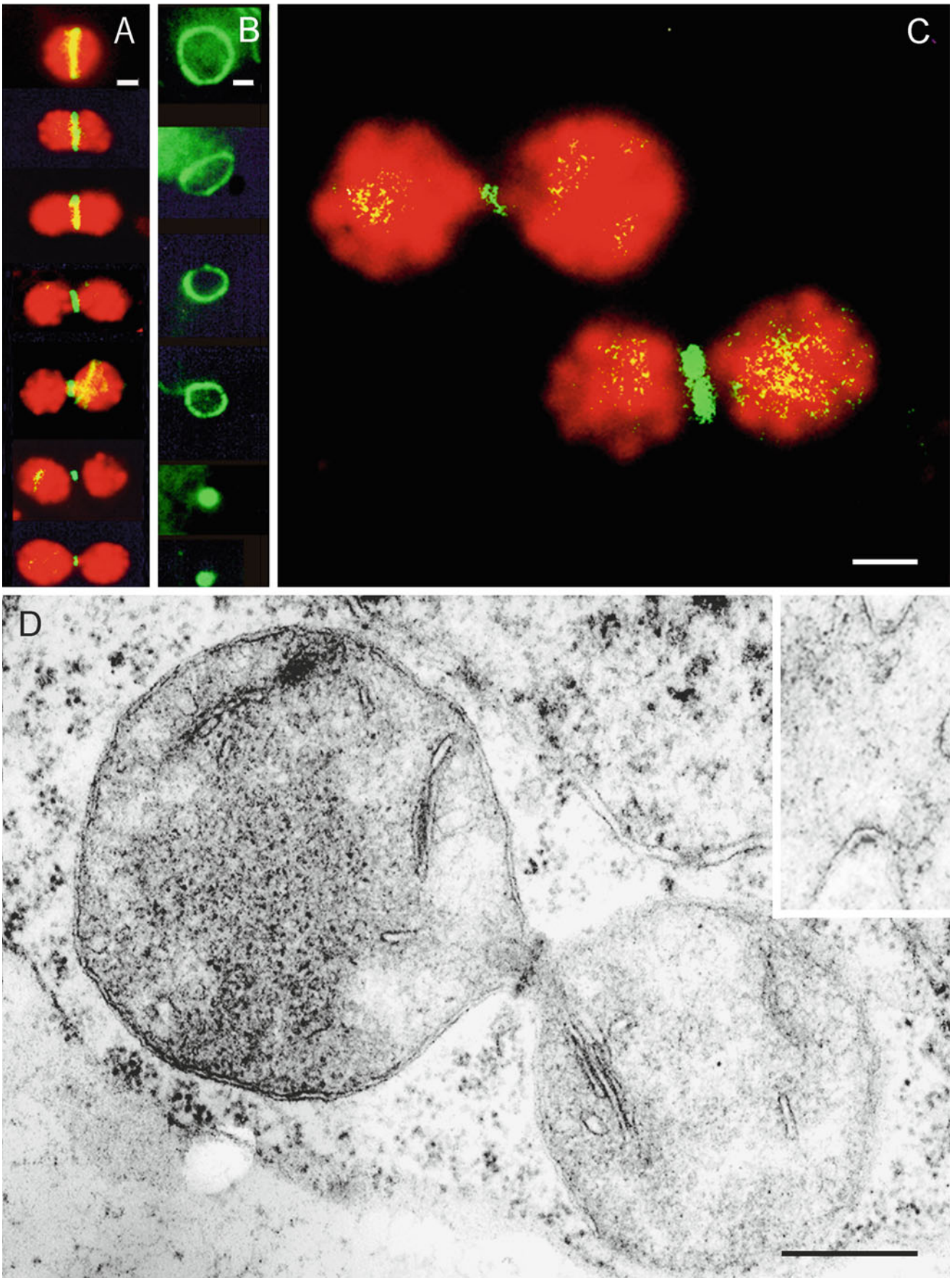
A
B
C
D

Plate 3.12

Active digestion of paternal chloroplast DNA in a young *Chlamydomonas reinhardtii* zygote

In most sexual eukaryotes, chloroplast (cp) and mitochondrial (mt) genomes are inherited almost exclusively from one parent. Uniparental inheritance of cp/mt genomes was long thought to be a passive result of the fact that eggs contain numerous organelles, while male gametes contribute, at best, only a few organelles and therefore little cp/mtDNA. However, uniparental inheritance occurs in organisms that produce gametes of identical sizes (isogamous), implying that the process is likely to be more dynamic.

In *Chlamydomonas reinhardtii*, although the maternal (mating type plus, *mt*+) and paternal (mating type minus, *mt*−) gametes are of equal sizes and contribute equal amounts of cytoplasm to the zygote, only cpDNA from the *mt*+ parent is inherited due to active degradation of *mt*− cpDNA within 60 m after mating. The method by which *Chlamydomonas* selectively degrades *mt*− cpDNA has long fascinated researchers (for review [25]).

This figure represents the process of the preferential elimination of *mt*− cp nucleoids (cpDNA-protein complex: white arrow in A) in a living *C .reinhardtii* zygote. Zygotes were incubated with 1:2000 SYBR Green I solution for 5 min at room temperature. SYBR Green I staining can visualize dsDNA molecules as yellow–green fluorescence when excited by blue light. The red fluorescence shown is autofluorescence emitted from chlorophyll in chloroplasts. Cell nucleus is indicated by "N".

Young living zygotes stained by SYBR Green I (**A**). The left and right chloroplasts were derived from *mt*+ and *mt*− gametes, respectively. At this time point, both of the chloroplasts have almost equal numbers of cp nucleoids. The identical zygote after 10 min (**B**). Nucleoids from the *mt*− chloroplast completely disappear, whereas mitochondrial nucleoids (arrowheads) are still visible. The active disappearance of *mt*− cp nucleoids was a rapid process that commenced about 40 m after zygote formation and was completed within 10 min. It has been confirmed that *mt*− cpDNA molecules are degraded during the disappearance of *mt*− cp nucleoids by single cell analysis using optical tweezers, indicating that the rapid disappearance of *mt*− cpDNA is the basis of uniparental inheritance [26]. A mutant defective in the active digestion of *mt*− cpDNA, *biparental* (*bp*) *31*, was recently isolated and detailed analysis of this mutant revealed that uniparental inheritance of cpDNA is strictly controlled by the *mt*+ gamete-specific homeotic gene, *GAMETE SPECIFIC PLUS* (*GSP*) *1* [27]. Scale bar: 5 μm. This figure is adapted from [25].

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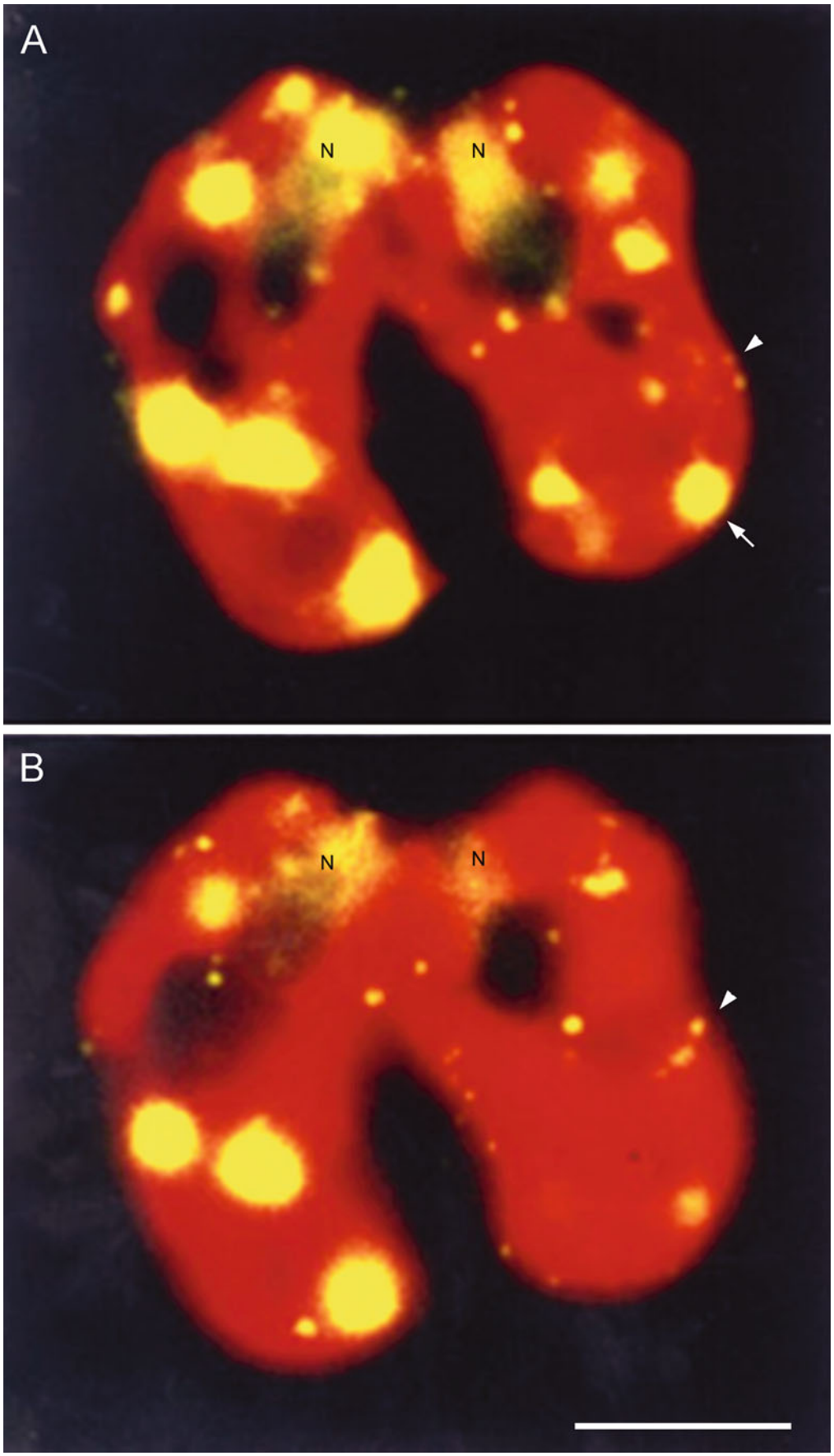
A
N
N
B
N
N

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