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Light-Regulated Translocation of Cytoplasm in Green Alga *Dichotomosiphon*

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Chloroplasts and other cytoplasmic granules in the freshwater coenocytic green alga *Dichotomosiphon tuberosus* streamed bidirectionally along the longitudinal axis of its tubular body. In response to light stimuli, the organelles migrated toward the apical regions and accumulated there. In the dark, they migrated toward the basal regions and stayed there. The cytoplasmic streaming and the light-regulated movement were inhibited by the presence of 5×10^{-4} M colchicine, but not by 100 μg/ml cytochalasin B. Local illumination with blue light caused reversible accumulation of the organelles in the illuminated zone. Single arrays of microtubules were found in the ectoplasmic layer of the alga, and both single and bundle arrays in the endoplasm. The endoplasmic microtubules disintegrated when the alga was treated for 24 h with colchicine. The involvement of the microtubules in the motive force generation for the bidirectional streaming and translocational movement of the organelles is discussed.

Key words: Cytoplasmic streaming — *Dichotomosiphon* — Light-dependent translocation (cytoplasm) — Microtubule.

Cell organelles are capable of moving in random or oriented fashion in response to internal and/or external stimuli. Their movements are important for the function and development of plant cells as well as animal cells. Light-induced movement of the chloroplasts is one such reaction. A single chloroplast in *Mougeotia* (Haupt 1959) or *Selaginella* (Mayer 1964) turns to either the face or profile position depending on the light intensity. In *Lemna* (Zurzycki 1962) or epidermal cells of *Vallisneria* (Seitz 1967), the chloroplasts migrate to the cell walls perpendicular to the direction of illumination (epistrophe) with low-intensity light stimulus but translocate to the side walls (parastrophe) with high-intensity light. In mesophyll cells of *Vallisneria*, the chloroplasts together with the cytoplasmic granules begin to stream rotationally along the side walls in response to light stimulus (Takagi and Nagai 1985). When *Bryopsis* (Mizukami and Wada 1981) or *Vaucheria* (Blatt and Briggs 1980) is illuminated locally, the chloroplasts accumulate in the illuminated zone. In the developing thallus of *Caulerpa* (Dawes and Barilotti 1969) or *Acetabularia* (Koop et al. 1978), the tip becomes white in the dark as a result of basipetal movement of the chloroplasts, which, during the day, are more concentrated in the upper parts of the thallus. *Ulva* (Britz and Briggs 1976) shows a circadian rhythm of chloroplast orientation.

Such movements are considered to result from successive processes of the intracellular systems, which are assumed to be comprised of signal sensing, signal transducing capable of regulating the movement, and actual movement system to change chloroplast distribution.

Major efforts have been made to analyze the sensing mechanism and identify the photoreceptor. Phytochrome is reported to be a very plausible photoreceptor because of the sensitivity

Abbreviations: CB, cytochalasin B; DMSO, dimethylsulfoxide.

to red and far-red light of the chloroplast movement in *Mougeotia* (Haupt 1959, Haupt and Schönbohm 1970), *Mesotaenium* (Haupt and Thiele 1961) and mesophyll cells of *Vallisneria* (Takagi and Nagai 1985). In other species, including the strong light response of *Mougeotia* chloroplast (Schönbohm 1963), the movements are sensitive to blue light and the reactions may be mediated by flavoprotein (Haupt 1982).

As one of the motile systems, the actin-myosin system is known to be involved in providing the motive force for organelles and the cytoplasmic movement. In the characean internode, the rotational streaming of the endoplasm is driven by an active shearing force generated through interaction between F-actin attached to the chloroplast files on the cell cortex and myosin-like molecules in the endoplasm (for review, see Kamiya 1981). Bidirectional multistriated streaming in *Acetabularia* is also based on the actin-myosin system (Koop 1981, Nagai and Fukui 1981, Fukui and Nagai 1985). Microtubules, in addition to their role in mitosis and ciliary movement, are regarded as elements along which organelles are transported (for review, see Hyams and Stebbings 1979, Dustin 1984). The movement of pigment granules in chromatophores (Murphy and Tilney 1974, Beckerle and Porter 1982, 1983), fast axonal transport in neuron (Grafstein and Forman 1980), and the movement of cytoplasmic granules in protozoa (Roth et al. 1970, Travis and Allen 1981, Koonce and Schliwa 1985) and keratocytes (Hayden et al. 1983) are all thought to occur along microtubules. However, reports are rather limited on plant cells. In *Caulerpa* (Kuroda and Manabe 1983, Manabe and Kuroda 1983) and *Bryopsis* (Mizukami and Wada 1981), the organelle movements are associated with microtubules. Also, increasing evidence for the existence of cytoplasmic dynein-like molecules (Hisanaga and Sakai 1983, Pratt 1984) suggests that the other motile system, the tubulin-dynein system, provides the motive force for the organelle movements. On the other hand, a soluble protein translocator that induces movement of axoplasmic organelles on microtubules was recently isolated from the axoplasm of the squid giant axon. The protein, named kinesin, is distinct from myosin and dynein. Thus, the microtubule-kinesin system has been proposed as a novel type of force-generating systems (Vale et al. 1985).

Further studies are needed on almost all species to elucidate the mechanism of organelle orientation movements in response to external stimuli in terms of signal transduction and regulation of the movement system. As a first step of inquiry along this line, we studied the light-mediated orientation movement of the organelles in a freshwater green alga, *Dichotomosiphon*, and the movement system which is implicated with microtubules.

Materials and Methods

Test Organism—Dichotomosiphon tuberosus, a coenocytic freshwater green alga, was originally collected from a paddy field on Okinawa Island. It has since been cultured unialgally in our laboratory in a nutrient medium composed of 2.5 mM KNO_3, 0.5 mM $MgSO_4$, 0.2 mM K_2HPO_4, and 0.5 mM $CaCl_2$ with the minor components of 9 μM $FeCl_3$, 0.06 μM $CoCl_2$, 0.24 μM $CuSO_4$, 0.3 μM $(NH_4)_6Mo_7O_{24}$, 2 μM $MnCl_2$, 0.28 μM $ZnSO_4$ and 9.7 μM H_3BO_4 and 55 μM Na_2EDTA. The pH was adjusted to 7.6. The medium was sterilized by autoclaving. The alga was planted in an agar layer at the bottom of a Petri dish (9 cm in diameter and 5 cm high) filled with the culture medium. The alga elongated at each tip where dichotomous branching occurred after every 3–10 mm elongation and grew up to several centimeters for 2–3 weeks, until it turned into an entangled mass. Constrictions, not the real septum, were formed at every 2–3-mm intervals along the tubular body of the alga. Several segments 2–3 cm long cut from the outermost portion of the mother culture were transplanted into fresh medium once a month to keep the alga elongating. The culture was maintained on a light-dark cycle of 12 h light (2,000 lux from white fluorescent lamps, FL20S.PG, National, Kadoma) and 12 h darkness at 21 ± 1°C.

Specimen preparation for monitoring orientation movement or for electron microscopy—A segment, 1–2 cm long, which included two to three sets of dichotomous branches was cut from the mother culture at around the end of the light or dark period. Each was mounted on a glass slide with a coverslip using a small amount of vaseline at each corner. The air gap between the glass slide and the cover slip was filled with fresh culture medium. When necessary, chemicals were added to the medium. Each segment was placed in a Petri dish moistened with a piece of filter paper and kept under light or darkness at 23 ± 1°C. Light of 2,000 lux was supplied with a bank of 20 W fluorescent lamps.

Monitoring chloroplast orientation movement—The orientation movement of the chloroplasts was expressed as their translocation, which was observed by measuring the brightness of a specimen image on a monitor TV. The brightness is proportional to the amount of accumulated chloroplast (cf. Britz and Briggs 1976). The set-up for the monitoring system is shown in Fig. 1. A specimen was enlarged ($\times$110) through a light microscope (Optiphot, Nikon Optics, Tokyo) with an 10$\times$objective lens and a video camera system (WV-1550, National, Kadoma) on a monitor TV (WV-5410, National, Kadoma). The light intensity on the monitor TV was measured with a photosensor (silicon photodiode S 1227-66BQ, Hamamatsu Photonics, Hamamatsu). The sensory face was fixed at a square of 6 mm which corresponds to 80% of the diameter of the enlarged specimen. The photocurrent from the sensor was recorded, through a current voltage converter (prepared by us using a Teledyne 1026), on a recorder (VP-652B, National, Kadoma). The intensity of the light transmitted through the specimen (I_c) and that without the specimen (I_0) were measured and the value, $1-(I_c/I_0)$, was expressed as the "relative absorbance." I_0 was adjusted to constant value at each time of measurement. After incubation of a specimen under defined conditions, the first measurements of intensity were made for a selected segment of the specimen at several positions at fixed distances from a branching position of the segment (cf. the position of the photosensor in Fig. 1). Subsequent measurements were made using the same segment at the same positions. The positions were determined using a scale on the TV screen. The segment on the microscope stage was longitudinally moved from the branching position to the apex.

For local illumination, the image of a small hole in a black plastic plate which was placed

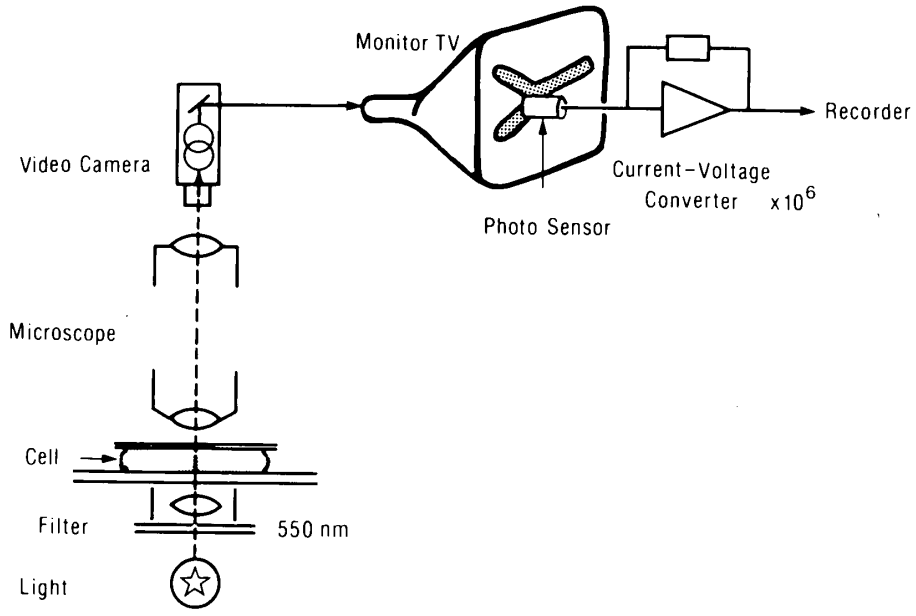

Fig. 1 Schematic diagram of the system for recording chloroplast translocation. For details, see text.

between the filters and the microscope field lens was focused with the condenser lens on the central plane of the cell. Blue light (λ=450 nm, 3×10^{18} photons/m^2·sec) was obtained by combining an interference filter (KL-45, Toshiba Electronics, Kawasaki) and a cut-off filter (Y-44, Toshiba Electronics, Kawasaki). Green light (λ=550 nm, 2.2×10^{17} photons/m^2·sec), as safe light for the observation, was also obtained by combining an interference filter (KL-55) and a cut-off filter (O-54). The intensity of the monochromatic light was measured with a silicon photodiode (S 1337-1010BQ, Hamamatsu Photonics, Hamamatsu), which was positioned in the focal plane of the light microscope. The output from the photodiode was recorded for later calculation of the quantum number.

Electron microscopy—Specimens were prefixed with 2% glutaraldehyde and 1% tannic acid buffered with 35 mM cacodylate (pH 7.0) for 2 h, under the conditions used to store the specimens before they were transferred to the fixative. At 15–20 min after immersion, the specimens in the fixative, the segments to be subjected to microscopy, were cut from the mother specimens. After washing twice with the buffer, the segments were postfixed for 1 h with 1% OsO_4 in the same buffer. After dehydration through a graded series of ethanol, they were transferred to Spurr's (1969) medium, then cut smaller, 2–3 mm long, to accelerate the resin penetration. Thin sections were made on a LKB Ultrotome. Thin sections were stained with uranyl acetate and lead citrate.

To observe negatively stained microtubules, the cytoplasm was squeezed out into a solution composed of 10 mM EGTA, 1 mM $MgCl_2$, 1 mM GTP, 2 mM DTT, 180 mM sorbitol and 50 mM PIPES buffer (pH 6.9). After gentle mixing, a drop of the mixture was placed onto a grid, then negatively stained with 2% aqueous solution of uranyl acetate. Observations were made with a JEM 100-C or a JEM 100-S type electron microscope at 80 kV.

Chemicals—Chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). CB from Aldrich Chemical Co. (Milwaukee, U.S.A.) was dissolved in DMSO and diluted with the culture medium; the final DMSO concentration was 0.5%. Colchicine from Sigma Chemical Co. (St. Louis, U.S.A.) was directly dissolved in the culture medium. Both were applied by gentle irrigation between the coverslip and the slide.

Results

Light-dependent orientation movement of the chloroplasts—In the tubulous coenocyte of *Dichotomosiphon tuberosus*, the chloroplasts and other cytoplasmic granules streamed along the longitudinal axis. The streaming pattern was multistriate (Kamiya 1962) and its direction was bidirectional, i.e., acropetal and basipetal. The streaming rate was 2–4 μm/sec. When the algal body was illuminated, movement of the chloroplasts and other cytoplasmic granules toward the apical end increased and the organelles accumulated gradually from the apex toward the basal end while maintaining basipetal movement, which could be confirmed in area where accumulation was scarce. The apical regions, therefore, appeared deeper green than the other regions of the alga. In the dark, the organelles migrated more toward the basal end and became concentrated there. The apical regions became lighter green as a result of the chloroplast evacuation. Fig. 2 shows a typical light-microscope view of an apical region of the specimen after 24 h incubation in light (L) and dark (D). In the light specimen, the apical region was occupied by a conspicuous amount of chloroplasts, most of which were still. Therefore, hardly any streaming of the cytoplasmic granules was observed in the region. However, streaming was seen in areas of constriction and dichotomous branching. In the dark specimen, most of the chloroplasts had evacuated from the apical region, where a small number of granules moved in a usual pattern. A small area close to the apex was sometimes hedged off from the usual translocational movement. The area remained greenish or transparent after incubation in dark or light, respectively.

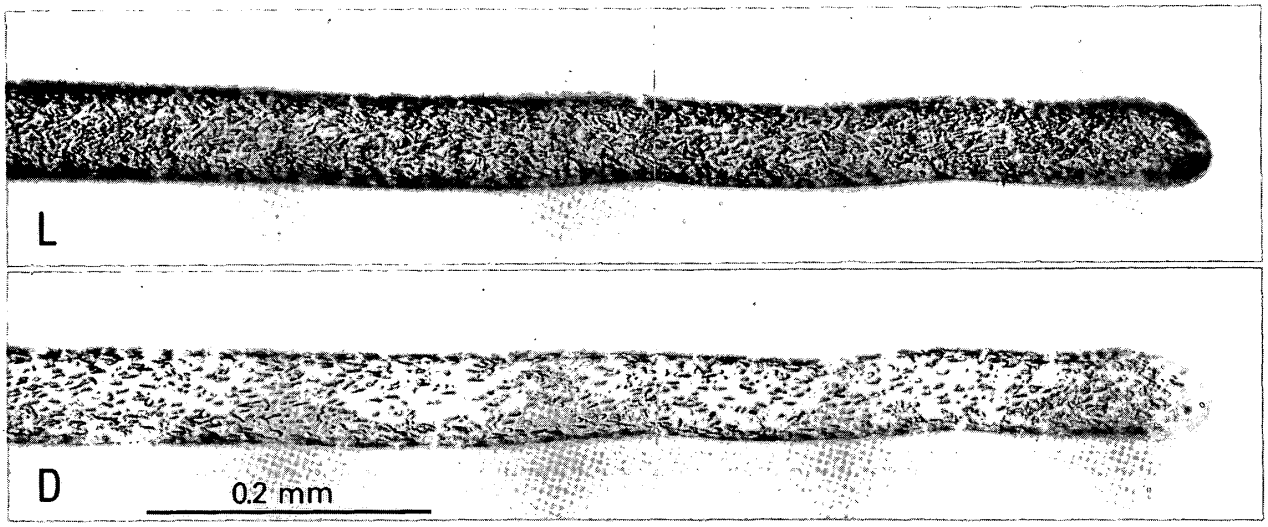

Fig. 2 Light microscopy image of the apical region of a specimen after 24 h incubation in light (L) and dark (D).

Fig. 3 shows electron-microscopic images of cross sections from such areas. Fig. 3(a) and (b) were obtained from a specimen conditioned similar to Fig. 2 (L). Cross section (a) was obtained from the region at 10–15 μm and (b) from the region at 100–105 μm from the apex of the specimen. Fig. 3(c) and (d) were obtained from a specimen conditioned similar to Fig. 2 (D). Cross section (c) was from a region corresponding to (a), and that of (d) to (b). Clearly, under the light, the region closest to the apex is filled with the cytoplasmic matrix intermingled with many small vesicles and nuclei, and rather a limited number of chloroplasts. The more basipetal region, shown in Fig. 3(b), is filled with a much larger number of chloroplasts together with nuclei, mitochondria and the cytoplasmic matrix. In the dark, a large portion of each corresponding region [shown in Fig. 3(c) or (d)] is replaced with a central vacuole and the remaining portion is occupied by a thin layer of cytoplasmic matrix in which the organelles are scattered. We will call the reaction which occurred in response to light and dark "light-dependent orientation movement" or "translocational movement."

When a small area of the specimen which had been kept in the dark was illuminated, migration of the chloroplasts together with the small cytoplasmic granules was observed under the light microscope. They migrated into the illuminated area from both apical and basal ends, when the area was far from the apex of the specimen as shown in Fig. 4. When the illumination was limited to the apical region, migration of the organelles from the basal end increased. The organelles first became trapped in a single layer, then gradually accumulated there while the illumination was maintained. When the light was turned off, the accumulated organelles moved out bidirectionally or basipetally within a period of time which depended upon the amount of which had accumulated. Thus, the accumulation was strictly light-dependent.

To examine the accumulation by electron microscopy, a serial cross section from the specimen locally illuminated (cf. Fig. 4) was made along the long axis of the specimen. One of the results is shown in Fig. 5. Section (a) was obtained from the region which was just outside the illuminated area and facing the apex of the specimen. Section (b) was from the middle region of the illuminated area and (c) from a region similar to (a) but facing the basal end. Clearly, the illuminated area is fully occupied by a conspicuous number of chloroplasts with many other organelles and the cytoplasmic matrix. However, the regions facing the apex and the base had a smaller number of organelles and a lesser volume of the cytoplasmic matrix. Instead, the large central vacuole can be seen. To express these phenomena quantitatively, the portion occupied

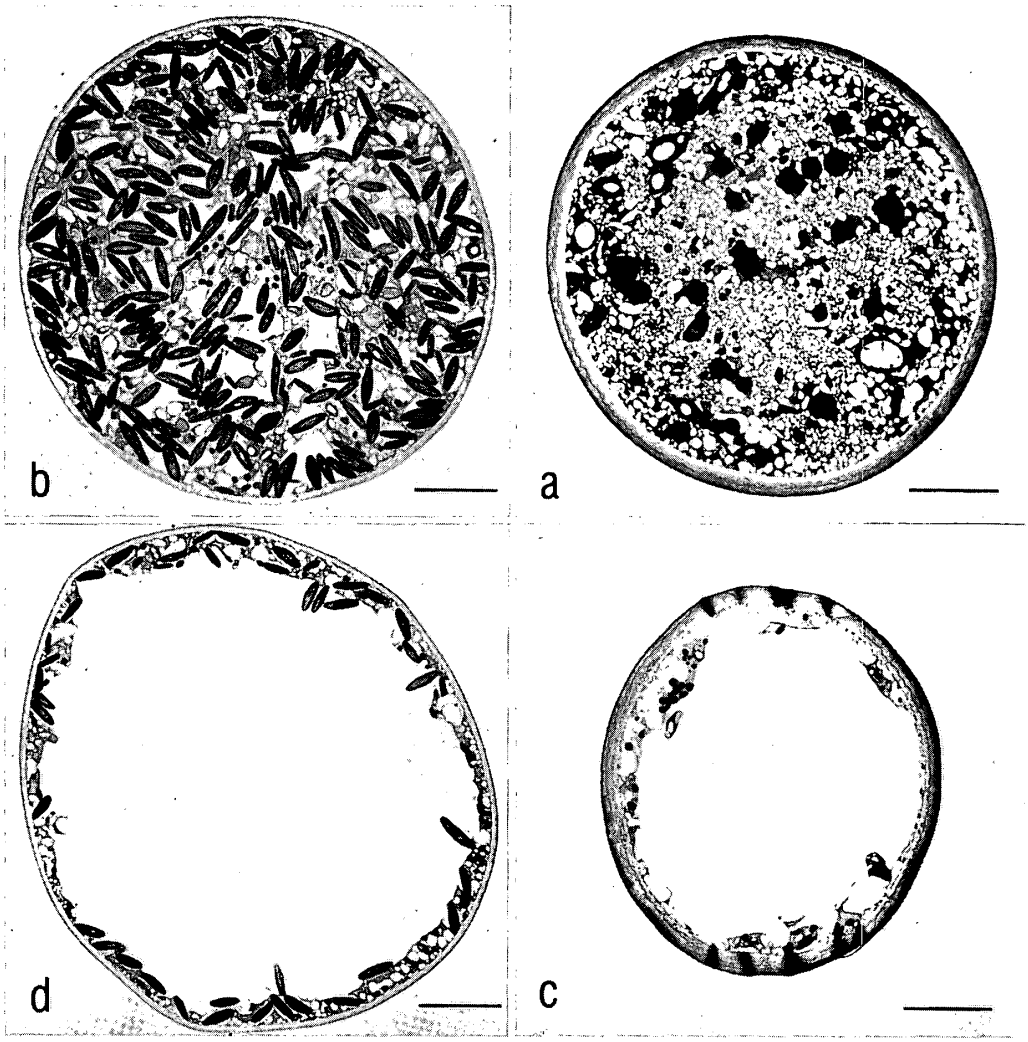

Fig. 3 Electron micrograph of cross sections from apical regions of specimens. Cross section (a) was obtained from the region at 10–15 μm and (b) at 100–105 μm to the apex of specimens kept in the light for 24 h. Cross section (c) was from the region corresponding to (a), and (d) to (b). Sections (c) and (d) were obtained from specimens kept in the dark for 24 h. Bar=10 μm.

by all inclusions, except fairly large vacuoles, was removed from each electron micrograph and weighed. These values, as relative volumes, were plotted against the long axis of the specimen (Fig. 6, –●–). The number of chloroplasts in each corresponding micrograph was also calcu-

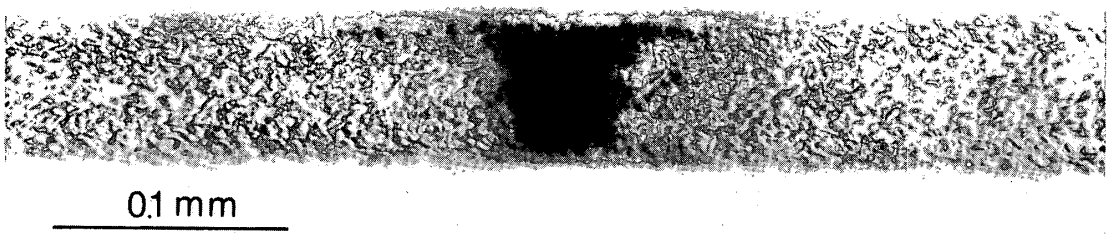

Fig. 4 Cytoplasm accumulation with local illumination.

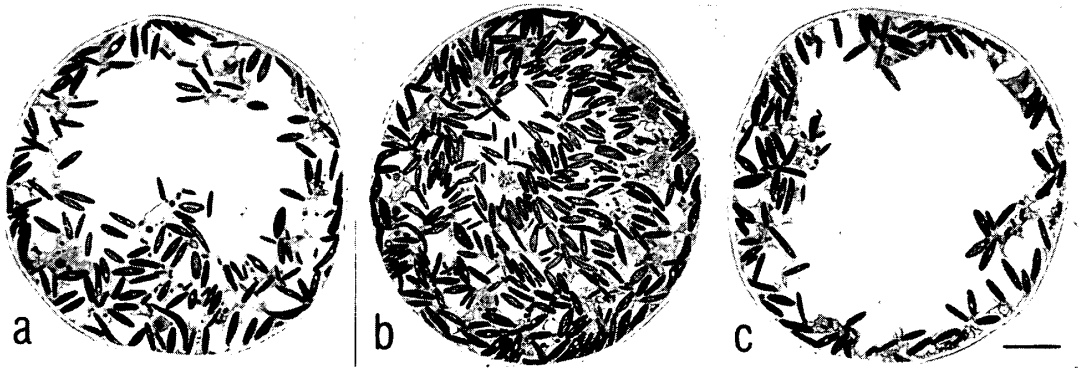

Fig. 5 Electron micrograph of a serial cross section of an illuminated area. Section (a) was obtained from the region just outside the illuminated area and facing the apex, (b) was from the middle region of the illuminated area and (c) was from a region similar to (a) but facing the basal end of the specimen. Bar=10 μm.

lated and plotted (Fig. 6, -□-). The figure shows that the accumulation is sharply limited within the illuminated area (shown by a rectangle) and that the number of chloroplasts which accumulated in each sectioned area agrees, in its relative value, with the amount of the inclusions accumulated there, suggesting that the chloroplasts migrate together with the other organelles and the cytoplasmic matrix.

The accumulation was most sensitive to blue light (λ=450 nm) and far less sensitive to green or red light (data not shown).

Motile system for orientation movement of the chloroplasts—To clarify the motile system, recordings were made of chloroplast translocation which took place in response to light and dark, and the effects of CB or colchicine on the translocation were examined. The translocation was expressed as changes in the distribution pattern of the chloroplasts along the long axis of the specimen. Fig. 7 shows a representative example of the translocation occurring normally in light and dark. The abscissa of the figure represents the length in mm from the dichotomous branching (shown by 0), where every measurement was started, to the apex of the selected segment (cf. Methods).

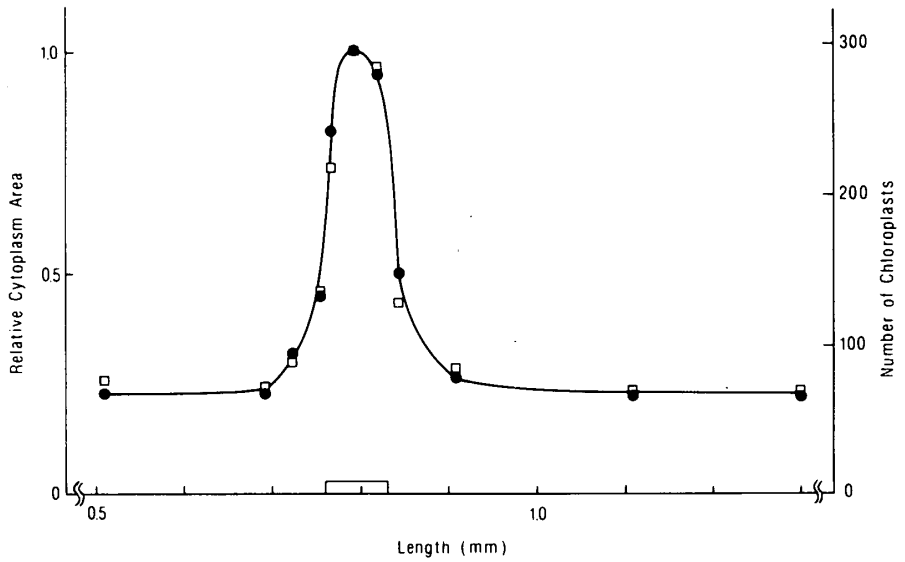

Fig. 6 Relative volume of the cytoplasm (-●-, left ordinate) and the number of chloroplasts (-□-, right ordinate), calculated from serial cross sections of a locally illuminated area. The abscissa represents the length in mm from the apex (left) to the basal end (right). For details, see text.

The ordinate represents the relative absorbance which is related to the number of existing chloroplasts where the measurement was made. The record shows that many chloroplasts accumulate after the first incubation in the dark (–●–) in the region near the branching. Lesser accumulation occurs toward the apex where the relative absorbance is less than 0.5. After the second incubation under light for 24 h (–○–), the chloroplasts which had accumulated in the basal region migrated toward the apical region. After the third incubation in the dark (–■–), the chloroplasts translocated again toward the basal region, and the fourth incubation under the light brought on their normal migration toward the apex (–□–). The four marks on the ordinate represent the cytoplasmic clog which formed when the specimen was cut away from the mother culture. The clog remained at its original position throughout the experiment. The figure also shows that the segment elongates about 1 mm during the period (96 h) of the recording.

A similar recording in the presence of CB at 100 μg/ml is shown in Fig. 8. Chloroplast translocation was not inhibited. The multistriate and bidirectional movements of the organelles were normal, indicating that the microfilaments may not play a part in driving the organelles.

Colchicine at 5×10^{-4} M was added after the first recording of chloroplast distribution in a segment which had been kept in the dark for 24 h (Fig. 9a, –●–). Then the specimen was kept further in the dark because the drug needed more than several hours to take effect. The distribution pattern after this period of incubation remained unchanged (–■–) and further incubation in the light did not cause changes in the chloroplast distribution pattern (–○–), indicating that their translocation was completely inhibited by the drug. An experiment was done with the same protocol but in the absence of the drug. In the second incubation under the dark condition (Fig. 9b, –■–), further migration toward the basal region proceeded so that the

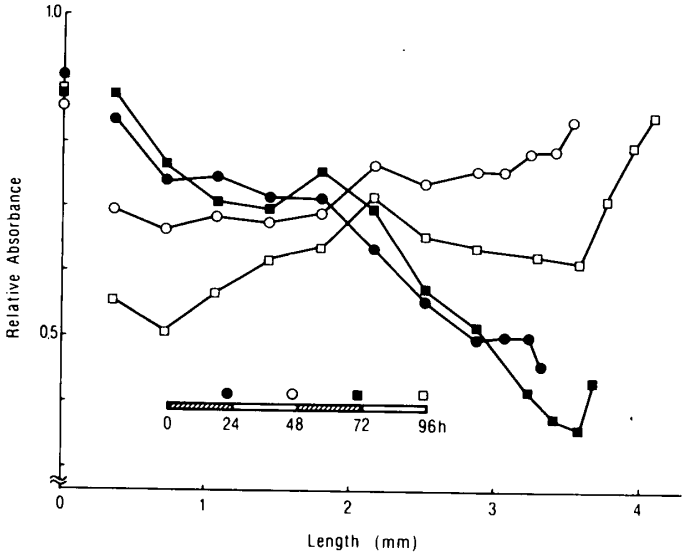

Fig. 7

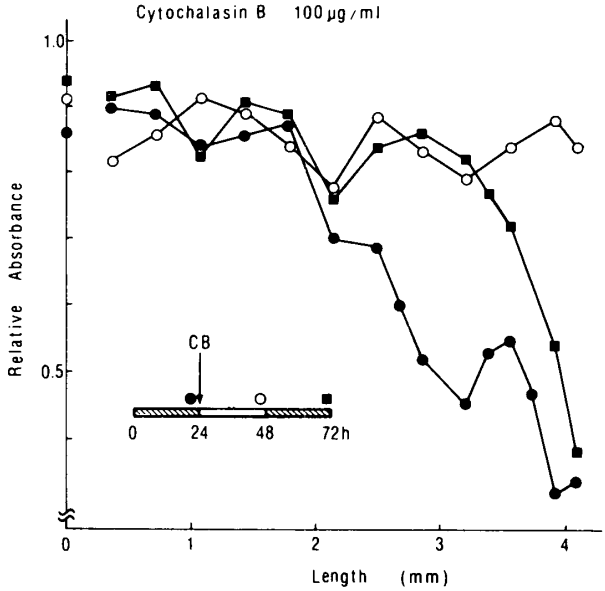

Fig. 8

Fig. 7 Recording of chloroplast translocation occurring normally in response to light (open rectangles) and dark (shadowed rectangles). The abscissa represents the length in mm from the dichotomous branching (shown by 0), where every measurement was started, to the apex of a specimen. The ordinate represents the relative absorbance which is correlated with the number of chloroplasts present at the place where the measurement was made. Marks on the ordinate represent the chloroplasts anchored around the region near the branching. Measurements were made after every 24 h of dark (closed marks) and light (open marks).

Fig. 8 Recording of chloroplast translocation in the presence of cytochalasin B at 100 μg/ml.

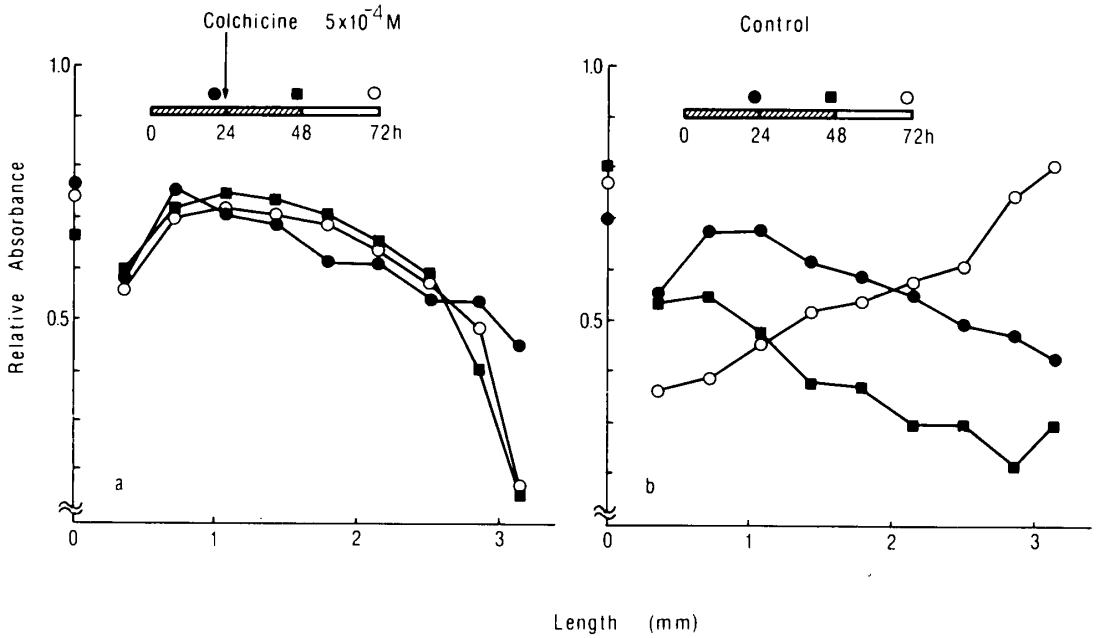

Fig. 9 a: Recording of chloroplast translocation in the presence of colchicine at 5×10^{-4} M. Colchicine was added to a specimen after 24 h incubation in the dark. The specimen was further kept in the dark for 24 h, then exposed to the light. b: Recording of chloroplast translocation in the absence of colchicine. The experimental protocol was the same as that of a.

relative absorbance as a whole decreased. After the subsequent incubation in the light (–○–), the chloroplasts migrated normally toward the apex.

Colchicine was also added to the specimen which had been kept under the light for 24 h (Fig. 10a, –○–), then kept further in the light. Although slight migration of the chloroplasts

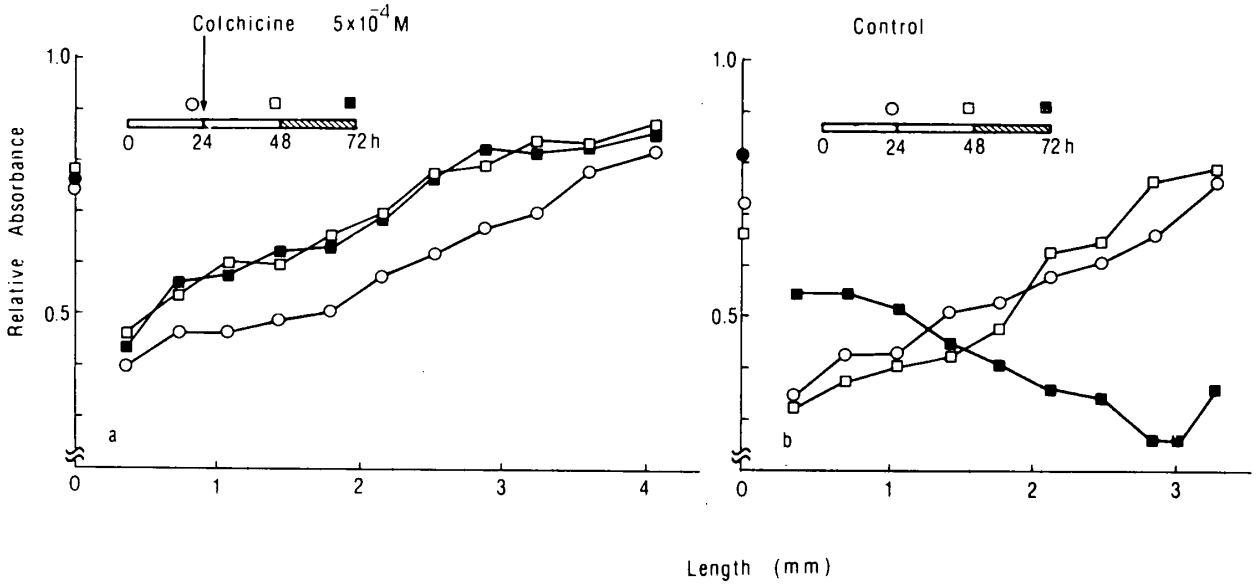

Fig. 10 a: Recording of chloroplast translocation in the presence of colchicine at 5×10^{-4} M. Colchicine was added after 24 h incubation in the light, and the specimen was kept in the light for 24 h before being brought to the dark. b: Recording of chloroplast translocation obtained under the same protocol as a, but in the absence of colchicine.

proceeded during the second light period (Fig. 10a, –□–), the migration toward the basal region in the following dark condition was completely inhibited (Fig. 10a, –■–). Organelle streaming was also inhibited. The precise location of the cytoplasmic striations along which the organelles had been moving became indiscernible. In the control specimen, on the other hand, the usual reactions were observed (Fig. 10b). These results suggest that the microtubules play an indispensable role in chloroplast translocation and that translocation toward both the basal and apical regions is associated with the microtubules.

Microtubules in the cytoplasm—The configuration and distribution of microtubules in the cytoplasm were examined for specimens illuminated for 12 h. Cross sections were obtained from regions around 200 μm from the apex of each specimen. Fortunately, as the diameter of the cell is about 60 μm, all microtubules could be surveyed in the cross sections not trimmed by the grid meshes. Microtubules, mostly in single array, could be seen all along the peripheral layer of the cytoplasm near the cell membrane. There were 22–42 peripheral microtubules, with an average of 31. The distance from their neighbors was not constant. The longitudinal axis of each microtubule agreed with that of the cell. At the inner portion of the cytoplasm, i.e., in the endoplasm, both single and bundle arrays of microtubules were seen with no regularity of location. Microtubules in a single array were counted to be 30. Bundle arrays were 32. The values were averages from seven cross sections. Each bundle was composed of 2 to 30 microtubules. A cross section image of a bundle is shown in Fig. 11. Each microtubule, ca. 25 nm in diameter, was composed of globular subunits and was surrounded by a clear electron

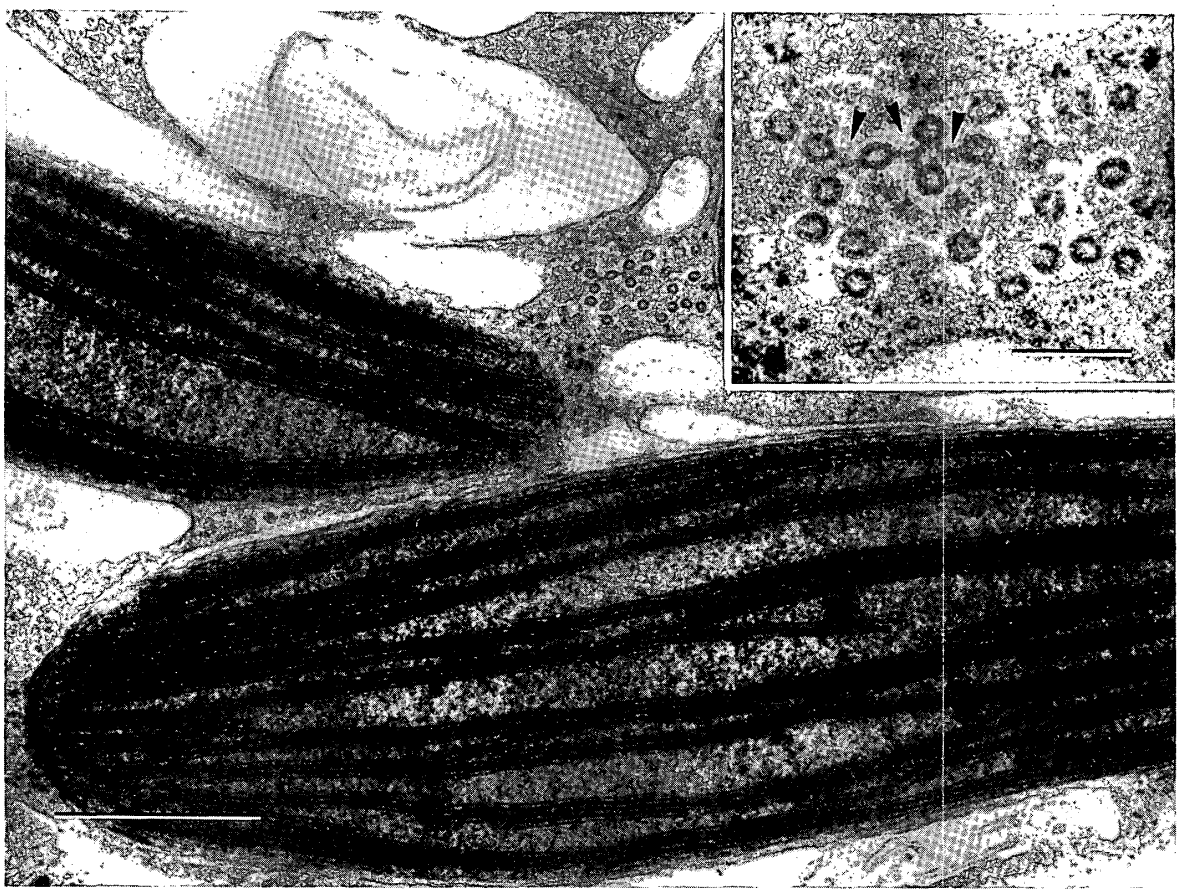

Fig. 11 Cross-section image of a bundle composed of 21 microtubules. Each microtubule, ca. 25 nm in diameter, is composed of globular subunits and is surrounded by a clear electron matrix. Some are linked to each other by cross bridges (inset, arrows). Bar=0.5 μm. Bar in inset=0.1 μm.

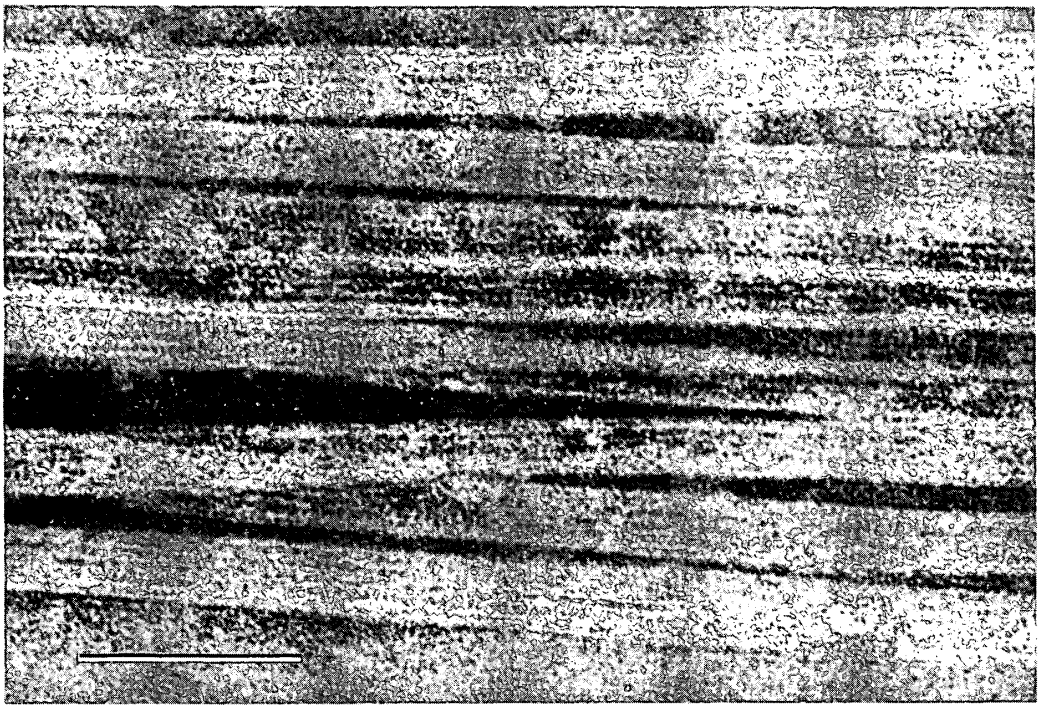

Fig. 12 A negatively stained bundle of microtubules in the squeezed cytoplasm. Globular subunits and protofilaments can be clearly seen. Bar=0.1 μm.

matrix. Sometimes cross-bridges linking neighbors could be seen (Fig. 11 inset, arrows). Microtubules were observed adjacent to the tonoplast, chloroplasts, mitochondria and nuclei. They were all oriented parallel to the longitudinal axis of the cell. Fig. 12 shows a bundle of

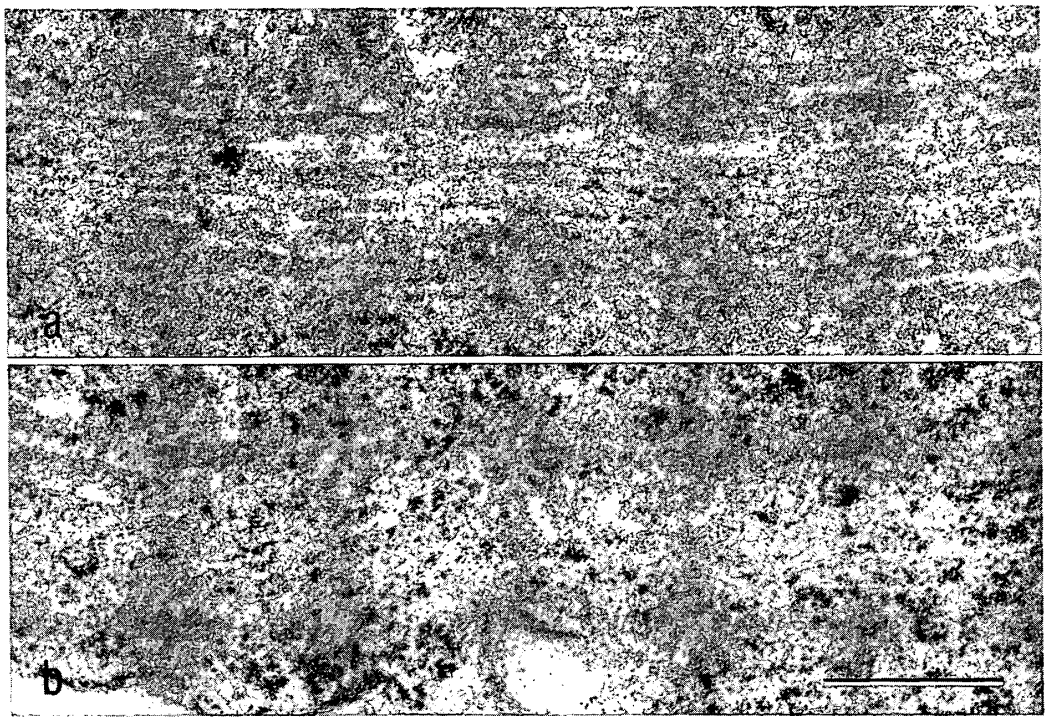

Fig. 13 Longitudinal section image of microtubules observed in the cytoplasm (a) and in the cytoplasm treated with colchicine at 5×10^{-4} M for 6 h (b). Bar=0.2 μm.

microtubules negatively stained. Each microtubule is very straight and subunits can be clearly seen.

In specimens treated with colchicine for 6 h (Fig. 13b), the microtubules were not straight and not regularly arranged as in normal cases (Fig. 13a). In specimens treated longer, they were not recognizable but dotted structures were observed in the areas where the microtubules were supposed to have been, suggesting their disintegration due to the binding ability of colchicine to tubulin dimer.

Discussion

The translocational movement was strictly light-dependent and directionally oriented. The chloroplasts migrated, together with other organelles such as nuclei and mitochondria and the cytoplasmic matrix, toward the apical end under the light and toward the basal end in the dark. The light-dependency of the translocation was supported by the fact that they migrated and accumulated in a limited area during the limited period of illumination.

The effect of colchicine on the translocation of the organelles and ultrastructural studies suggest that microtubules may be directly involved in providing the motive force for bidirectional and translocational movements of the organelles. Ultrastructural studies revealed two types of microtubules in their location and array. One was in the ectoplasmic layer of the cell and mostly in a single array (peripheral microtubules). The other was in the moving endoplasm. The peripheral microtubules are thought to be for the cytoskeleton and/or for the arranging of microfibrils in the cell wall (for review, see Gunning and Hardham 1982). Microtubules in the endoplasm (cytoplasmic microtubules) may contribute to the motive force generation. The occurrence of organelle movement in association with single microtubules has been reported in the giant amoeba, *Reticulomyxa* (Koonce and Schliwa 1985), and an in vitro system (Schnapp et al. 1985). Microtubules in bundles as motile elements have been found in *Caulerpa* rhizome (Manabe and Kuroda 1983). It is not clear whether microtubules in a single array can really play a role in the motive force generation in this alga. If the motive force can be produced in a single array, the bundle array would be a less efficient system. Thus, the co-existence of single and bundle arrays of microtubules must occur due to some physiological necessity which is unknown at present.

The polarity orientation of microtubules is important for understanding the microtubule-based motility. Studies on several organisms have shown that bidirectional movement of organelles can take place in the presence of microtubule arrays in which the vast majority of the microtubules have uniform polarity (Euteneuer and McIntosh 1981, Allen and Borisy 1974, Haimo et al. 1979, Telzer and Haimo 1981). If this is the case in this alga, *Dichotomosiphon*, the usual bidirectional movement of the organelles is thought to occur in association with the cytoplasmic microtubules. For the light-dependent translocation, an additional mechanism, such as microtubule rearrangement and/or trapping of the organelles at the apical or the basal region in response to light or dark, would be necessary. Precise examination of microtubule distribution in each specimen illuminated or kept in the dark is in progress to find the answer to this question. It is known that tip-growing organisms generate an endogenous electrical current such that the positive charge flows into the apex and exits from the trunk (for review, see Nuccitelli 1983). If Ca ion carries this current to some extent and the current changes light-dependently, the Ca ion concentration in the apical region should be affected the most. Under such circumstances, the microtubules in the apical region would probably be assembled or disassembled. Furthermore, changes in Ca ion transport across intracellular membrane system(s) in the apical region would contribute to microtubule rearrangement. Contribution from factor(s) other than Ca ion would also occur. A locally illuminated specimen should be

usable as a model system for examining microtubule rearrangement and the organelle trapping mechanism.

The involvement of dynein-like molecules in microtubule-associated motility has been suggested in some kinds of cells (Clark and Rosenbaum 1982, Kuroda and Manabe 1983, Pratt 1984). Gilbert et al. (1985) showed that vesicles isolated from the giant axon of squid moved in both directions on a single dynein-free flagella axoneme in the presence of ATP and further that the vesicles were associated with the microtubules via cross-bridging filaments. They suggested that the cross-bridging filaments projecting from the vesicle surface may be the most plausible cause of vesicle movement. In our specimen, the involvement and mode of involvement of dynein-like molecules or of other ATPase candidates remain to be investigated. Linkages of microtubules with chloroplasts, nuclei or mitochondria have not yet been observed. It is also not clear whether such motile vesicles found in squid axons really exist and they are equipped with ATPase to slide actively on microtubules. In Characeae, it was observed that motile endoplasmic organelles equipped with globular bodies, which are supposed to be composed of myosin molecules (Nagai and Hayama 1979), slide actively on bundles of microfilaments to drive the endoplasm.

Interaction of microtubules with the putative dynein or some other protein translocator is thought to be regulated by some factor(s), which may have its mode of action modified by light. This system also should be involved in the mechanism of light-dependent organelle translocation.

Preliminary observations suggest that translocation of the cytoplasm to apical or basal regions and organelle accumulation in locally illuminated areas are most sensitive to blue light and far less sensitive to green or red light. The same pigments as those acting in the light-induced chloroplast movement and viscosity change in the cytoplasm of *Vallisneria* epidermal cells (Seitz 1967) and those in the chloroplast accumulation by local illumination induced in *Bryopsis* thallus (Mizukami and Wada 1981) may also act in the signal-sensing mechanism of this alga.

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