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LIGHT-CONTROLLED CYTOPLASMIC STREAMING IN *VALLISNERIA* MESOPHYLL CELLS

SHINGO TAKAGI

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Abbreviations

APW= artificial pond water

ATP= adenosine 5'-triphosphate

DMSO= dimethyl sulfoxide

EGTA= ethyleneglycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid

P_{fr}= phytochrome, far-red light absorbing form

P_r= phytochrome, red light absorbing form

PIPES= piperazine-N,N'-bis(2-ethanesulfonic acid)

Abstract

Light regulating mechanism of rotational cytoplasmic streaming in Vallisneria mesophyll cells was investigated. First, the effect of monochromatic light irradiation on the streaming was examined. Red light (650 nm, 1.9 W/m^2) was most effective to induce streaming. Other monochromatic lights of the same quantum number ($0.6 \times 10^{19}/s \cdot m^2$) had much lower effect. Although the induced streaming was markedly inhibited specifically by far-red light (730 nm, 1.2 W/m^2) irradiation, this inhibitory effect did not appear in the presence of La^{3+}, a Ca^{2+}-channel blocker.

Second, changes in the distribution of calcium in the cytoplasm was demonstrated by electron microscopic cytochemistry. In the mobile cytoplasm under red light irradiation, little precipitate was formed in the reaction with antimony. On the other hand, a large amount of precipitate was visible in the cytoplasm rendered immobile by far-red light irradiation. However, this increase in the amount of precipitate was not observed in cells which were irradiated with far-red light in the presence of La^{3+}. These suggested that red and far-red lights controlled the streaming by changing Ca^{2+} concentration in the cytoplasm.

Third, intracellular Ca^{2+} concentration regulating the streaming was estimated. Leaf segment was incised in the middle of the mesophyll cell layers and the exposed mesophyll cells were treated with solutions of various Ca^{2+} concentrations in the

presence of a calcium ionophore A23187. Streaming was induced or maintained in solutions which contain Ca^{2+} at lower than 10^{-6} M. In contrast, Ca^{2+} at higher than 10^{-5} M had a definite, inhibitory effect on the streaming.

Finally, protoplasts were prepared enzymatically from mesophyll cells to assay Ca^{2+}-fluxes across the cell membrane. Ca^{2+} concentration in a test solution bathing the protoplasts was measured as an absorbance change of Ca^{2+}-sensitive dye murexide by spectrophotometry. Red light irradiation increased Ca^{2+} concentration of the test solution and subsequent far-red light irradiation produced a rapid decrease down to the dark control level. Vanadate (10 μM), which is known as a Ca^{2+}-ATPase inhibitor, inhibited the red-light induced Ca^{2+} efflux from the protoplasts.

The results are summarized as follows; 1) Cytoplasmic streaming in Vallisneria mesophyll cells is induced when Ca^{2+} concentration in the cytoplasm decreased lower than 10^{-6} M, and ceases when it increases higher than 10^{-5} M, 2) Red light induces streaming by decreasing Ca^{2+} concentration in the cytoplasm and far-red light inhibits the streaming by the opposite way, 3) Red light activates Ca^{2+} efflux from the cells and far-red light reverses the effect. The involvement of phytochrome as a photoreceptive pigment is discussed.

Introduction

In leaf cells of the aquatic angiosperms, Elodea and Vallisneria, rotational streaming of the cytoplasm is induced by external stimuli such as irradiation with visible light (= photodinesis) or application of various chemicals (= chemodinesis). This type of streaming is called "induced" or "secondary" streaming, while that seen in characean internodal cells is called "spontaneous" or "primary" streaming because it persists under natural conditions (cf. Kamiya 1959).

There has been a pile of studies on chemodinesis and they have described a wide range of chemicals which have an ability to induce the streaming (Kamiya 1959). For example, some of amino acids show the effect at a concentration as low as 10^{-8} M. Heavy metals such as manganese, magnesium and copper are also capable of inducing the streaming, but their effects have been interpreted to be pathological. In spite of a number of efforts, the working hypothesis to explain these facts consistently has not been established yet.

Regarding photodinesis, many of the predecessors also have worked actively. Among others Seitz (1967) determined the action spectrum for the cyclosis of chloroplasts in Vallisneria epidermal cells. From the results of experiments using metabolic inhibitors, he has further proposed that the physiological effect of light is due to a change in the availability of ATP which is supplied from oxidative and photosynthetic phosphorylation; thus an increased availability of ATP by light irradiation induces the

streaming (Seitz 1971).

It is well established that various kinds of cell motility are based on interaction between actin and myosin. In the case of Elodea and Vallisneria, 1) the microfilament bundles which are composed mainly of F-actin are found in the ectoplasmic layer in Vallisneria epidermal cells (Yamaguchi & Nagai 1981) and also in mesophyll cells (Takagi & Nagai 1983), 2) cytochalasin B inhibits the streaming in Elodea canadensis (Forde & Steer 1976) and in Vallisneria mesophyll cells (Ishigami & Nagai 1980), 3) a myosin-like protein is extracted and partially purified from Egeria densa (= Elodea densa) (Ohsuka & Inoue 1979). These suggest that the streaming is driven by the actomyosin system.

A number of reports have indicated that Ca^{2+} controls many kinds of cell movements as well as cytoplasmic streaming in plant cells. Forde & Steer (1976) examined the effect of calcium chelating agents on streaming in Elodea. They suggested that Mg^{2+} is required for maintenance of streaming and that Ca^{2+} has an inhibitory effect. Yamaguchi & Nagai (1981) observed that streaming in Vallisneria epidermal cells was induced by application of EGTA. An inhibitory effect of Ca^{2+} at higher than 10^{-6} M on the streaming was reported in characean cells (Hayama et al. 1979, Tominaga & Tazawa 1981, Williamson & Ashley 1982). Also in Vallisneria, we already showed that treatment of mesophyll cells with EGTA induced streaming, and that the induced streaming was inhibited by subsequent far-red light irradiation. By electron microscopic cytochemistry, we found that calcium content in the cytoplasm decreased when cells were treated with

EGTA and increased after far-red light irradiation (Takagi & Nagai 1983). These observations have made us suppose that streaming in Vallisneria mesophyll cells is regulated by a change in Ca^{2+} concentration in the cytoplasm, and that light irradiation exerts its effect on the streaming through changing Ca^{2+} concentration in the cytoplasm.

In several kinds of plant cells, Ca^{2+} fluxes across the cell membrane are controlled by light irradiation (Dreyer & Weisenseel 1979, Hale & Roux 1980, Das & Sopory 1985, Wayne & Hepler 1985). Ca^{2+} is supposed to act as a "second messenger" who mediates between light perception and activation of cell functions. In this thesis, I aim to elaborate on the following three points; 1) Effect of light irradiation on the cytoplasmic streaming in Vallisneria mesophyll cells, 2) Intracellular Ca^{2+} concentration regulating the streaming, 3) Light-affected Ca^{2+} fluxes in the protoplasts prepared from the mesophyll cells.

Materials & Methods

Plant - *Vallisneria gigantea* Graebner was purchased at a tropical fish store and cultured in a water-filled glass basin with soil at the bottom. The culture was kept under a 12 h-light (2000 lux) and 12 h-dark regime at 18 - 20 °C. The light source was a bank of 20 W fluorescent lamps (FL·20S·PG, National, Kadoma, Japan).

Pretreatment of specimens - A leaf segment was cut from a healthy plant at the end of the light period and cut into smaller pieces about 2 mm long. After evacuation of the air trapped in the intercellular space, each piece was placed in a separate plastic vessel with 40 ml of APW (0.2 mM NaCl, 0.05 mM KCl, 0.1 mM $Mg(NO_3)_2$, 0.1 mM $Ca(NO_3)_2$ and 2 mM Tris-maleate buffer at pH 7.0), and incubated under the original light condition for 24 h. During the 24 h-incubation, APW was replaced once at the end of a dark period with fresh APW. After one cycle of dark and light, each piece was mounted on a glass slide with a coverslip using a small amount of vaseline at each corner, then the glass slide was immersed in a Petri dish filled with fresh APW and kept in the dark for another 12 h at 18 - 22 °C. After these procedures, the specimens could be used for the experiments without being touched with forceps. All the cells prepared in this way did not show any sign of streaming.

For the experiments applying a calcium ionophore to mesophyll cells, each leaf piece was further incised in the middle of the mesophyll cell layers and trimmed to 5 mm x 2 mm at

the beginning of the pretreatment. Opened specimen was placed on a glass slide with the mesophyll cells facing to an objective lense of a light microscope (Olympus, Tokyo, Japan), then was mounted as described above. After the following incubation under the original light condition of the 12 h-dark and 12 h-light regime, the specimen was kept in the dark for another 12 h in APW containing 0.1 % DMSO.

Irradiation with monochromatic light - The specimen was irradiated on the microscope stage through a condenser lens with monochromatic light (450 - 800 nm), which was produced with a combination of an interference filter (Toshiba, Kawasaki, Japan) with an appropriate cut-off filter (Toshiba, Kawasaki, Japan) as described in Table 1. These filters were placed in front of a tungsten lamp (Olympus, Tokyo, Japan) or a halogen lamp (Cabin, Tokyo, Japan). To eliminate heat effects, a water-filled glass tank was placed in the light path. The irradiation intensity was controlled with neutral density filters (Fuji Photo Film, Tokyo, Japan).

The intensity of each monochromatic light was measured with a silicon photodiode (S 1337-1010 BQ, Hamamatsu Photonics, Hamamatsu, Japan), which was positioned in the focal plane of the light microscope. The photodiode output was monitored on a voltmeter (SP-H5V, Riken Denshi, Tokyo, Japan). Quantum number was calculated assuming that the total energy transmitted through the interference filter represented the peak wavelength of the filter. When the specimen was irradiated with light of over 700 nm, green light (500 nm, 0.15 W/m^2) was used for observations

Table 1 Combination of filters to obtain monochromatic lights

Interference filter	Wavelength (nm)	Half-band-width(nm)	Cut-off filter
KL-45	445.5	12.5	Y-44
KL-50	495.4	14.1	Y-44
KL-55	553	15.5	O-54
KL-58	578	12.5	O-54
KL-60	595.5	16.5	O-54
KL-62	622	12.5	R-60
KL-65	650	17.5	R-64
	651.5	14.5	R-64
KL-68	678	13.5	R-64
KL-70	696	16.5	R-69
KL-73	729.4	14	R-69
KL-75	748.5	16	R-69
KL-80	796	13	R-69

because it had no effect on induction and cessation of the streaming.

Measurement of effectiveness - Specimens were examined with 20x objective and 10x ocular lenses. The effectiveness of light irradiation and chemical application on induction or cessation of the streaming was expressed as the ratio of streaming cells to total observed cells. About 50 - 100 cells from five to ten different leaf segments were examined for each test condition. The criterion for streaming cells was that the chloroplast(s) exhibited continuous and one-directional movement for at least 5 s.

Electron microscopy - To examine the intracellular localization of calcium, specimens were fixed in the presence of 2 % potassium pyroantimonate. The entire specimen was irradiated with light, and induction or cessation of streaming in all cells were examined before it was transferred to the fixative. Specimens were fixed for 2 h at 20 °C with 2.5 % glutaraldehyde buffered with 20 mM cacodylate at pH 7.0, under evacuation for 10 min at the beginning of the fixation. After washing twice with the buffer solution, the specimens were fixed for another 2 h at 20 °C with 2 % OsO_4 in the same buffer solution, under evacuation as before. They were embedded in Spurr's resin (Spurr 1969) after dehydration through a graded series of ethanol, and then sectioned. A JEM 100-S electron microscope was used at 80 kV.

Distribution of precipitate - Precipitate formed in cells under various conditions (see Results II) in the reaction with antimony was examined by electron microscopy. Every whole mesophyll cell

in randomly selected sections was divided into several parts and photographed. More than 50 micrographs including about ten cells were selected for each condition, and the outline of every structure in micrographs was traced on graph paper. The precipitate and the cytoplasmic matrix (the cytoplasm excluding chloroplasts, mitochondria and other cytoplasmic vesicles) were cut out with scissors, and their respective areas were determined by measurement of their weights. Distribution of the precipitate for each micrograph was estimated as [area of precipitate]/[area of cytoplasmic matrix].

Test solutions to control the intracellular Ca^{2+} concentration - A test solution was composed of 0.1 mM KCl, 0.1 mM $Mg(NO_3)_2$, 5 mM EGTA (Wako, Osaka, Japan), 10 μM A23187 (Sigma, St Louis, U.S.A.), 10 mM PIPES buffer (pH 7.0) and 10^{-8} - 10^{-4} M of Ca^{2+}. Free Ca^{2+} at 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M were prepared by adding 0.5, 2.0, 4.0, 4.5 and 5.5 mM of $Ca(NO_3)_2$, respectively. Each Ca^{2+} concentration was calculated according to the association constant between EGTA and Ca^{2+} in the presence of Mg^{2+} (Fabiato & Fabiato 1978). Concentration of DMSO in the solution was 0.1 %, which had no effect on the streaming.

The solution was applied to the specimen by gentle irrigation of the space between the coverslip and the glass slide. The volume ratio of each application to a specimen was larger than 150. All the experimental manipulations and observations were made under green light.

A stock solution of EGTA (200 mM) was made in distilled water with NaOH to ajust the pH to 7.0, then diluted with the

test solution to 5 mM before use. A23187 was dissolved in DMSO at 10 mM and stored in a freezer. The stock solution was diluted with the test solution to 10 μM before use.

Protoplasts preparation - Protoplasts were obtained from mesophyll cells to assay Ca^{2+} fluxes across the cell membrane. A leaf segment was cut from the mother culture at the end of the light period and cut into smaller pieces. After the incubation under the original light condition of the 12 h-dark and the 12 h-light regime, they were transferred to an enzyme solution containing 2 % Cellulase Onozuka R-10 (Yakult, Tokyo, Japan), 0.05 % Pectolyase Y-23 (Seishin Pharmaceutical, Tokyo, Japan), 1 % dextran sulfate (Meito Sangyo, Nagoya, Japan), 0.2 M NaCl, 0.5 mM $Ca(NO_3)_2$ and 10 mM Tris-maleate buffer at pH 5.5. Digesting condition was standing for 20 h in the dark at 20 °C followed by gentle shaking for 15 min. This condition left almost all the epidermal cells unreleased. The protoplasts from mesophyll cells were harvested by filtering the solution through a rough nylon mesh, and they were washed first with the enzyme solution lacking cellulase and pectolyase, and then with a test solution described after by centrifuging for each 2.5 min at about 70 x g. The viability of protoplasts was determined by observing their cytoplasmic streaming. Typical yield was 10^4 protoplasts from 4 g fresh weight of leaf segments.

Spectrophotometric measurements - After the washing, the protoplasts were introduced at the bottom of a measuring cuvette with a test solution which contained 50 μM murexide (Sigma, St Louis, U.S.A.), 1 % dextran sulfate, 0.2 M NaCl, 0.1 mM KCl, 0.1

mM $Mg(NO_3)_2$, 0.1 mM $Ca(NO_3)_2$ and 10 mM PIPES buffer at pH 7.0. The whole volume of the test solution including the protoplasts was 0.7 ml. Changes in the Ca^{2+} concentration of the test solution were monitored by measuring changes in the absorbance of murexide at 544 nm with a two-wavelength/double beam spectrophotometer Model 356 (Hitachi, Tokyo, Japan), the reference beam being set at 500 nm (Ohnishi 1978). The absorbance changes produced by cellular pigment(s) were not detected because the protoplasts were settled in the undermost layer just below where the measuring beams passed through the cuvette. The protoplasts, in the cuvette which was set at the cuvette holder of the spectrophotometer, were irradiated with an actinic light from above, while the sample chamber was opened and the measurements of the absorbance were intermitted. Red (650 nm, 0.4 W/m^2) and far-red (730 nm, 0.3 W/m^2) lights were produced with a combination of an interference filter with an appropriate cut-off filter as described before (Table 1).

Immediately after the introduction into the cuvette, the protoplasts were irradiated with far-red light for 10 min. 30 min after the end of the far-red light irradiation, the absorbance of murexide reached a constant value, and these protoplasts were used for Ca^{2+}-flux assay at 20° C. The test solution was made fresh daily because murexide was unstable in the water.

Treatment of protoplasts with 10 μM vanadate was accomplished by adding 7 μl of 1 mM sodium orthovanadate to the 0.7 ml of the test solution, and keeping the preparation for 30

min in the dark. This application had no effect on the absorbance of murexide.

Results

I: Effect of light irradiation on the streaming

Induction of streaming by monochromatic light irradiation - A *Vallisneria* leaf has several layers of mesophyll cells surrounded by a layer of epidermal cells (Fig. 1A). To minimize the disturbance of actinic light, only mesophyll cells near the leaf edges were used, because they are only two layers thick (Fig. 1B).

First, effects of monochromatic light irradiation of various wavelengths on streaming was examined to study precisely the mode of photodinesis in *Vallisneria* mesophyll cells. After pretreatment, the specimen was irradiated continuously with monochromatic light of a different wavelength. The fluence rate was a constant quantum number $0.6 \times 10^{19}/s \cdot m^2$. Several minutes after the initiation of light irradiation, agitation of the cytoplasmic particle became vigorous and some of them made local streamlets. The chloroplasts moved intermittently at first, then assembled linearly and began to move continuously. The time course of this process differed according to the wavelength of incident light. Once the cytoplasm streams stationarily, the nucleus, chloroplasts and cytoplasmic particles moved along the cell wall. The rate of streaming was more or less constant at 10 - 20 μm/s.

The number of streaming cells (N_x in Fig. 2) was counted at 5 - 10 min intervals. The ratio of N_x to the total observed cells (N_{total} in Fig. 2) was plotted as the percentage against

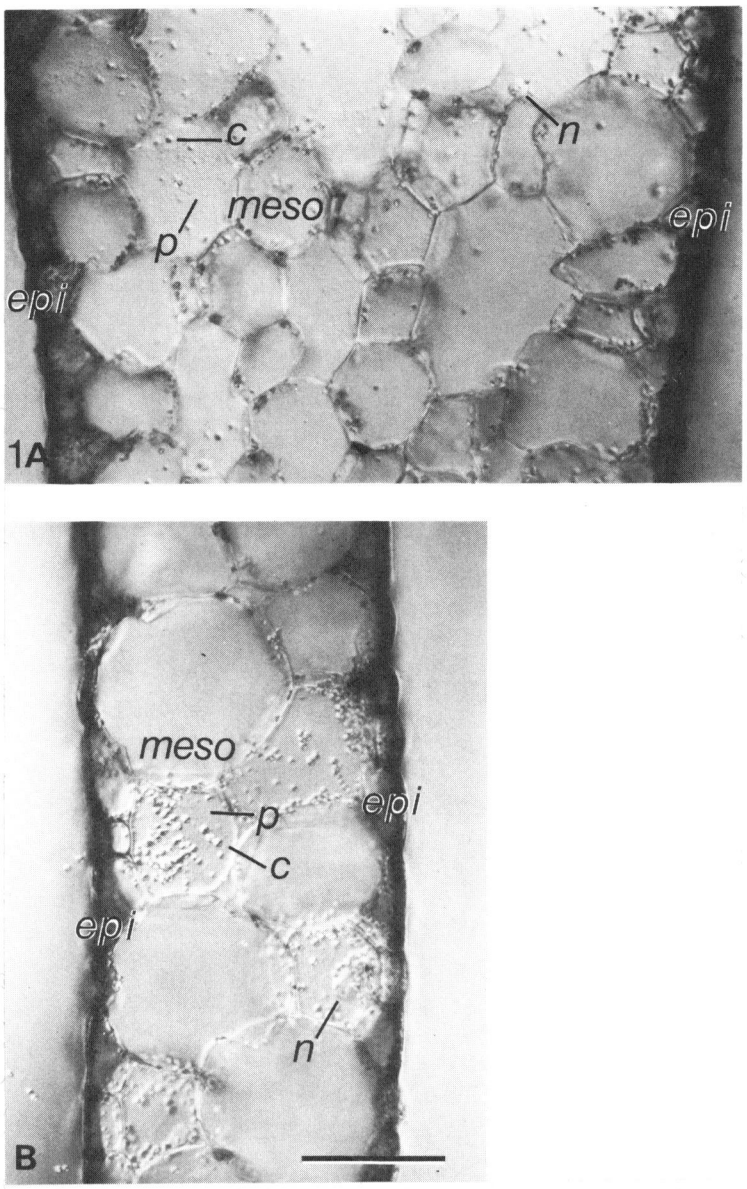

Fig. 1 Photomicrographs of cross-sections of Vallisneria leaf. A: A middle area where several layers of mesophyll cells (meso) are found between the two layers of epidermal cells (epi). B: An area near the leaf edge where only two mesophyll cell layers are found between the two epidermal cell layers. n= nucleus, c= chloroplast, p= cytoplasmic particle. Bar= 100 μm.

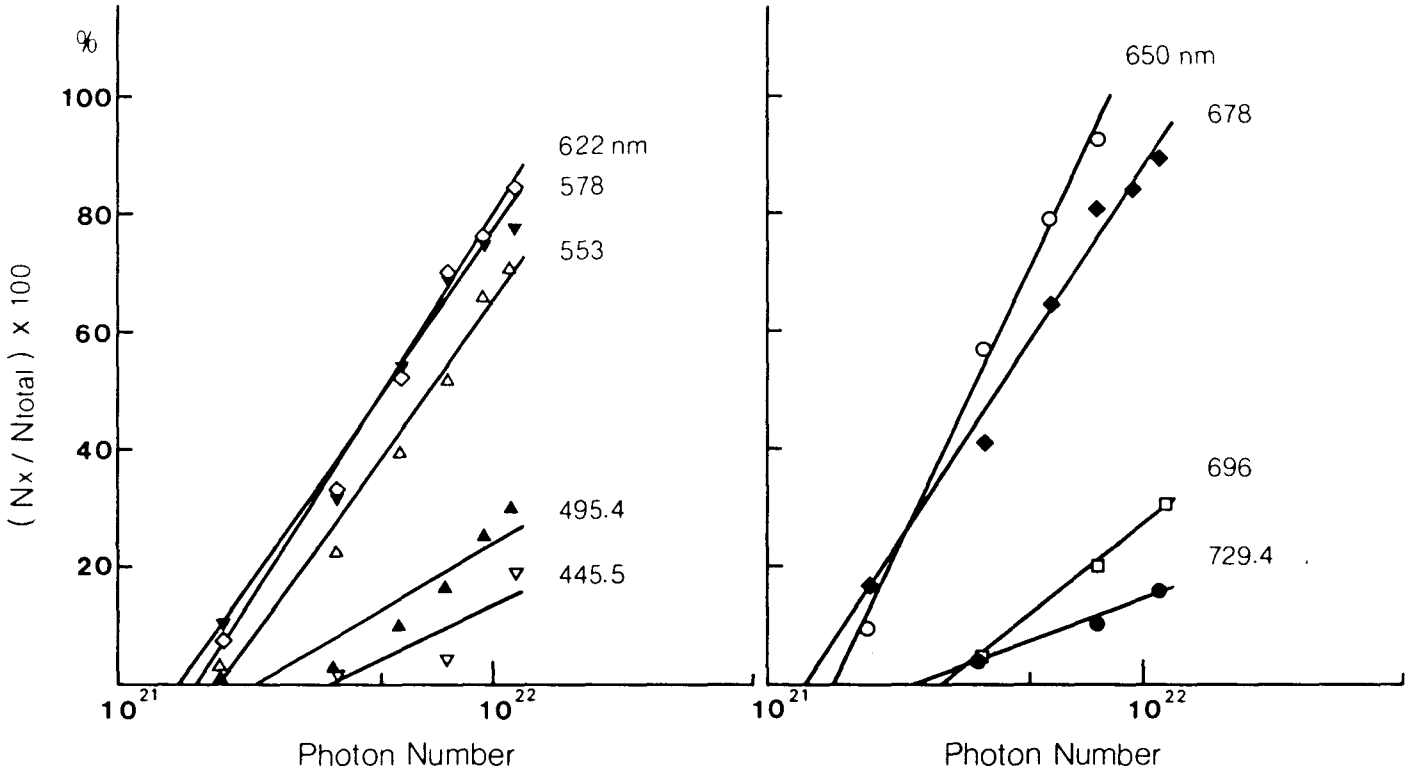

Fig. 2 Induction of streaming by monochromatic light irradiation. Specimens, in which cells exhibited no streaming after the pretreatment as described in Materials & Methods, were irradiated continuously with monochromatic light of various wavelengths. The fluence rate was a constant quantum number 0.6 x $10^{19}/s \cdot m^2$. The number of streaming cells (N_x) was counted at 5 - 10 min intervals, and the ratio of N_x to the total observed cells (N_{total}) was plotted as percentage against quantum number. About a hundred cells from ten different leaf segments were examined for each wavelength.

quantum number. The N_x/N_{total} ratio increased with time and had a linear relationship to the logarithm of quantum number (Fig. 2). Light of 650 nm (the fluence rate of a quantum number $0.6 \times 10^{19}/s \cdot m^2$ at this wavelength corresponds to 1.9 W/m^2) was most effective; the N_x/N_{total} ratio reached 90 % at 20 min and 100 % at 30 min of irradiation.

To show the wavelength dependency more clearly, the data in Fig. 2 were replotted in Fig. 3. The N_x/N_{total} values for a given wavelength at the quantum number that gave 50 % and 90 % N_x/N_{total} for 650 nm light were expressed as relative quantum effectiveness, N_x/N_{total} values for 650 nm light being 1.0. The effectiveness diminished as the wavelength became either shorter or longer than 650 nm.

Fig. 4 indicates that the N_x/N_{total} ratio for 650 nm light increased proportionally to the logarithm of irradiation time at fluence rates lower than 1.9 W/m^2. All the extrapolated points met at 2.5×10^2 s. Up until this time, streaming was not induced, and after that the N_x/N_{total} ratio increased depending on the intensity of incident light.

<u>Cessation of streaming caused by light irradiation</u> - Streaming induced by EGTA treatment in the dark was inhibited specifically by continuous far-red light irradiation (Takagi & Nagai 1983). In this study, I found that far-red light irradiation also inhibited the streaming induced by red light irradiation. Fig. 5 is a time course of cessation of streaming caused by far-red light (730 nm) irradiation. The fluence rate of a constant quantum number $0.4 \times 10^{19}/s \cdot m^2$ at this wavelength corresponds to

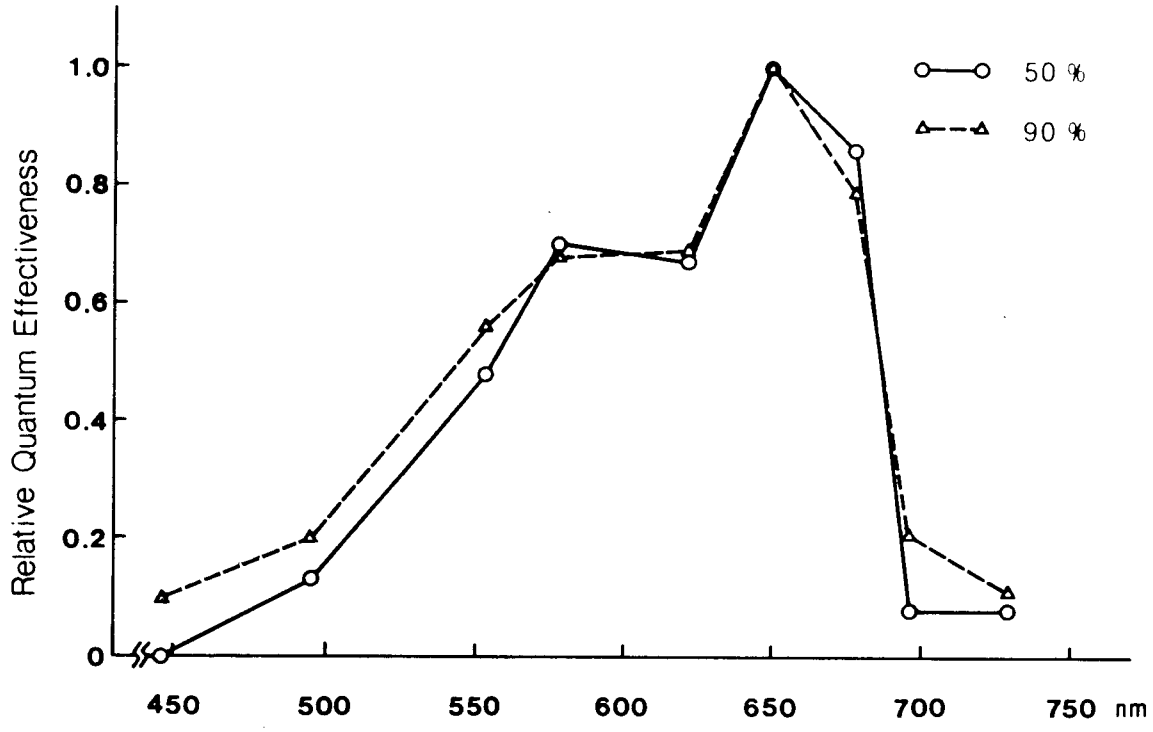

Fig. 3 Relative quantum effectiveness of monochromatic light on the induction of streaming. The N_x/N_{total} ratios for individual wavelength obtained from Fig. 2 at the quantum number that gave 50 % (-O-) and 90 % (--Δ--) induction for 650 nm light were plotted in relative values.

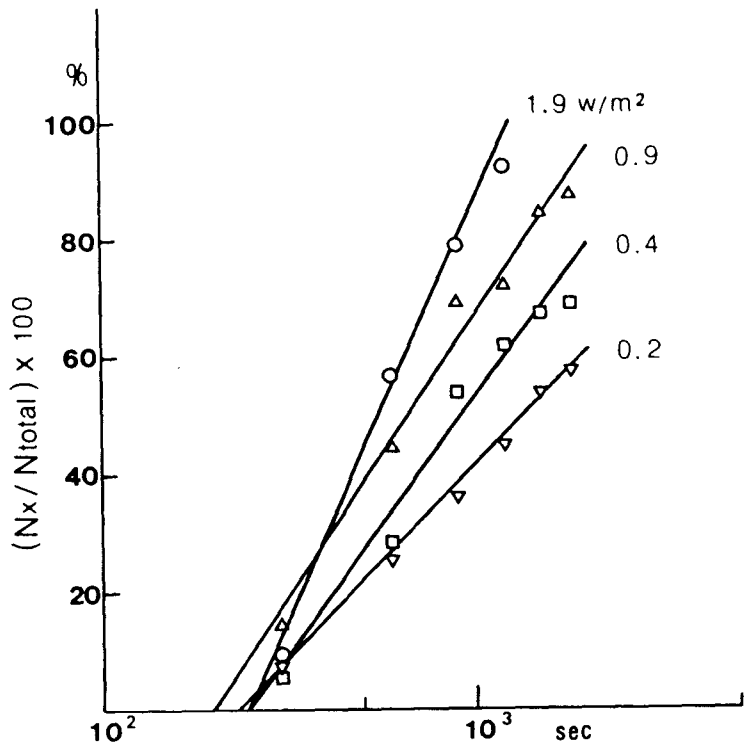

Fig. 4 Relationship between effectiveness in induction of streaming and irradiation time. Specimens after the pretreatment were irradiated continuously with light of 650 nm at a given intensity. The fluence rate of a quantum number 0.6 x $10^{19}/s \cdot m^2$ at this wavelength corresponds to 1.9 W/m^2. The N_x/N_{total} ratio is plotted as the percentage against irradiation time.

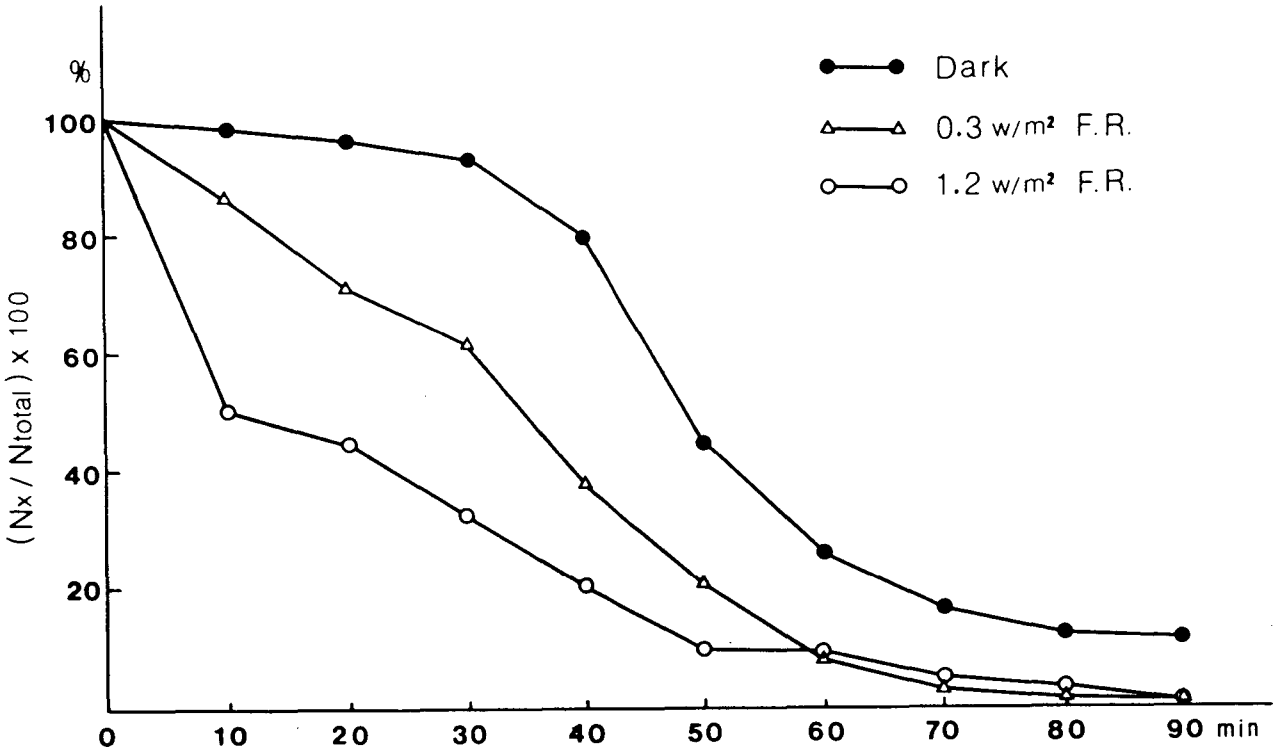

Fig. 5 Cessation of streaming caused by far-red light (730 nm) irradiation. After red light (650 nm, 1.9 W/m^2) irradiation to induce streaming in all cells, the specimens were either kept in the dark (-●-) or irradiated continuously with far-red light at 0.3 W/m^2 (-Δ-) and at 1.2 W/m^2 (-O-). The fluence rate of a constant quantum number $0.4 \times 10^{19}/s \cdot m^2$ at this wavelength corresponds to 1.2 W/m^2.

1.2 W/m^2. The dark control had a sigmoidal time course. It showed only a slight decrease of the N_x/N_{total} ratio in the first 10 min. In contrast, the streaming ceased in 50 % of the N_{total} irradiated at an intensity of 1.2 W/m^2. At 0.3 W/m^2, the N_x/N_{total} ratio decreased more gradually. It took 35 min to reach 50 % cessation. The effect of far-red light at a higher intensity (ca. 3 W/m^2) was almost the same as that of 1.2 W/m^2, and a lower intensity (ca. 0.1 W/m^2) had almost the same effect as that of 0.3 W/m^2 (data not shown).

Next, I investigated the effectiveness of monochromatic light irradiation on the cessation of red light induced streaming. After irradiation with red light (650 nm, 1.9 W/m^2) to induce streaming in all cells, the specimen was either irradiated continuously with monochromatic light (mono) or kept in the dark (dark). The fluence rate of monochromatic lights was a constant quantum number $0.4 \times 10^{19}/s \cdot m^2$. The N_x was counted at 10 and 40 min after monochromatic light irradiation and the results were expressed as relative quantum effectiveness ($[(N_x/N_{total})_{dark} - (N_x/N_{total})_{mono}]/(N_x/N_{total})_{dark}$) in Fig. 6, being normalized based on the N_x/N_{total} values in the dark. Far-red light had a definite and accelerating effect on the cessation of streaming. The effectiveness slightly diminished as the wavelength became longer than 730 nm. Red and green lights had virtually no effect; the time course of N_x/N_{total} for red and green lights were almost the same as the dark control. The effect of far-red light was enhanced with time.

The inhibitory effect of far-red light was also produced in

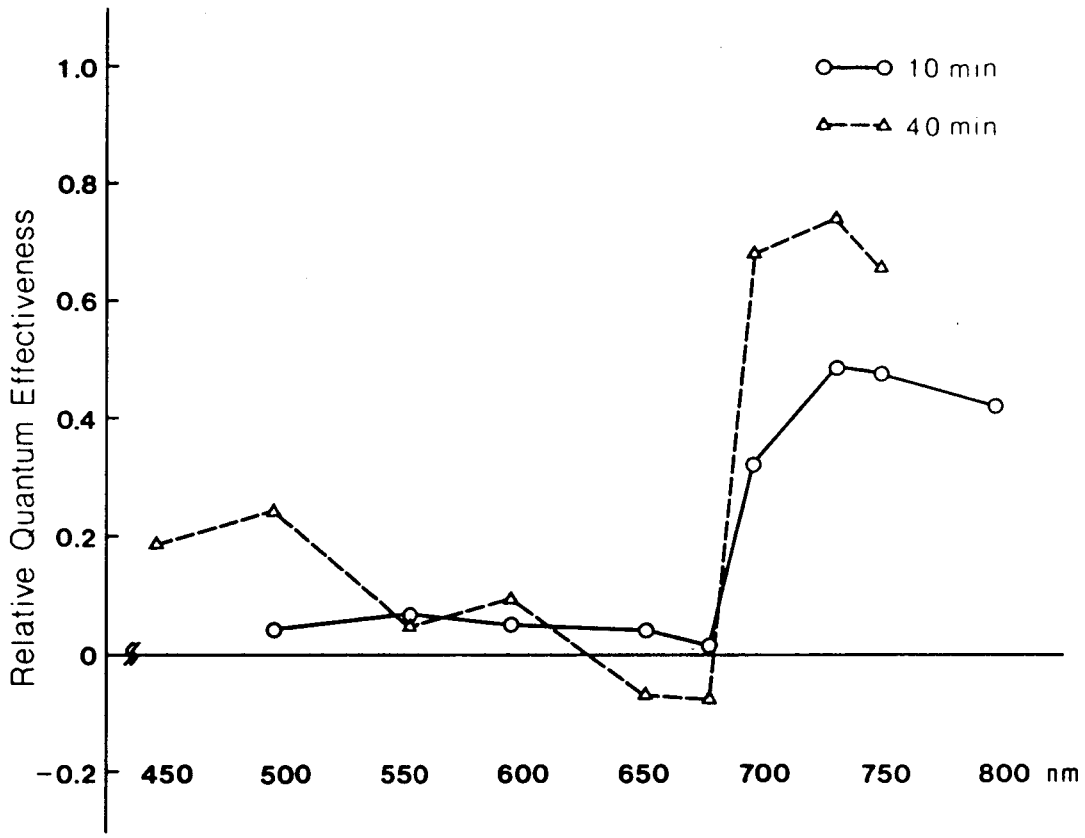

Fig. 6 Relative quantum effectiveness of monochromatic light on the cessation of streaming. After red light (650 nm, 1.9 W/m^2) irradiation, the specimen was either individually irradiated continuously with monochromatic light (mono) or kept in the dark (dark). The fluence rate of monochromatic lights was a constant quantum number $0.4 \times 10^{19}/s \cdot m^2$. The N_x was counted 10 min (-O-) and 40 min (--Δ--) after monochromatic light irradiation. Relative quantum effectiveness was expressed as $[(N_x/N_{total})_{dark} - (N_x/N_{total})_{mono}]/(N_x/N_{total})_{dark}$.

pulse irradiation. After continuous red light (650 nm, 1.9 W/m^2) irradiation to induce streaming in all cells, the specimen was irradiated with far-red light (730 nm, 1.2 W/m^2) for 1 min and kept in the dark for 10 min. As shown in Table 2, the N_x/N_{total} ratio decreased to 31 % during the dark period. However, this effect was reversed by the subsequent red light (650 nm, 1.9 W/m^2) irradiation for 1 min immediately after the far-red light irradiation. The streaming ceased in only 14 % of the N_{total}. This reversibility in the reaction was repeatedly observed.

Effect of La^{3+} on the streaming - Streaming was induced by EGTA treatment, and an inhibitory effect of far-red light on the streaming decreased to almost the dark control level when calcium was excluded from the external medium (Takagi & Nagai 1983). Ca^{2+}, which was transported from the outside into the cell seemed to cause the cessation of streaming. To clarify the involvement of an influx of Ca^{2+}, an effect of La^{3+}, a Ca^{2+}-channel blocker (Lettvin et al. 1964), was examined. There have been some reports that showed exogenously applied La^{3+} inhibits Ca^{2+}-mediated responses (La Claire 1982, Wayne & Hepler 1984).

Specimens were irradiated continuously with red light (650 nm, 1.9 W/m^2) to induce streaming in all cells in 0.1mM-$LaCl_3$-containing APW. This concentration of La^{3+} neither induced streaming in the dark nor affected the induction of streaming by red light irradiation. The specimens were then divided into two groups. One was kept in the dark and the other was irradiated continuously with far-red light (730 nm, 1.2 W/m^2). Fig. 7 clearly shows that the inhibitory effect of far-red light did not

Table 2 Effects of 1 min light irradiation

1 min irradiation	N_x/N_{total} (%) after 10 min
FR	31.3
FR·R	85.7
FR·R·FR	51.5
FR·R·FR·R	71.4

R: λ_{max} = 650nm, 1.9W/m^2
FR: λ_{max} = 730nm, 1.2W/m^2

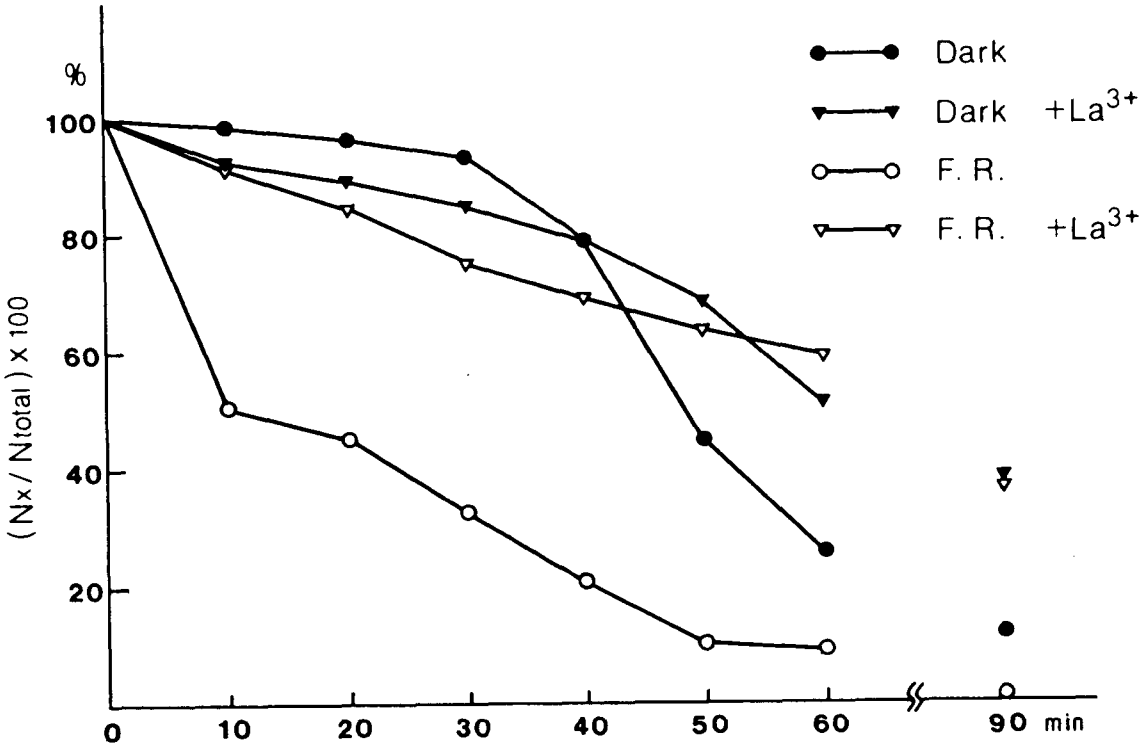

Fig. 7 Effect of lanthanum on the cessation of streaming. After red light irradiation in 0.1 mM-$LaCl_3$-containing APW, the specimen was either kept in the dark (-▼-) or irradiated continuously with far-red light (730 nm, 1.2 W/m^2)(-▽-). Solid circles and open circles are time course of the cessation of streaming in normal APW in the dark and caused by far-red light irradiation, respectively (see Fig. 5).

appear in La^{3+}-containing APW. The N_x/N_{total} ratio decreased only to 75 % even after 30 min, whereas 70 % of the cells in the absence of La^{3+} stopped their streaming by this time. In the dark, time course of the cessation of streaming in the first 30 min was almost same whether in the presence or absence of La^{3+}. The results indicated that the cessation of streaming was due to an influx of Ca^{2+} from the outside into the cell during far-red light irradiation.

II: Intracellular Ca^{2+} concentration and the streaming

Calcium in the cytoplasm - Excluding calcium from the extracellular medium (Takagi & Nagai 1983) or application of La^{3+} (Fig. 7) retarded the cessation of streaming caused by far-red light irradiation. These suggested that Ca^{2+} was transported from outside into the cell, and that an increase in Ca^{2+} concentration in the cytoplasm inhibited the streaming. To demonstrate changes of Ca^{2+} concentration with the light-controlled motility of the cytoplasm, I fixed cells in the presence of potassium pyroantimonate, a fairly specific precipitant of calcium. First, in specimens just after the pretreatment when the cytoplasm did not show any sign of streaming, I confirmed that precipitate was formed in the vacuole, the cytoplasm and the cell wall (Fig. 8a). Next, I fixed specimens that had been irradiated with red light to induce streaming in all cells. As shown in Fig. 8b, little precipitate was observed in the cytoplasmic matrix. There was a small amount of precipitate in the chloroplasts and at the border between the cytoplasm and the cell wall.

On the other hand, much more precipitate was visible in the cytoplasmic matrix when streaming was completely inhibited by far-red light irradiation after red light irradiation (Fig. 8c).

Table 3 quantitatively demonstrates these observations to some extent. At the end of each successive treatment, I examined the relative area of precipitate to the area of cytoplasmic matrix. These ratios fell into 0 to 0.4, and were divided into

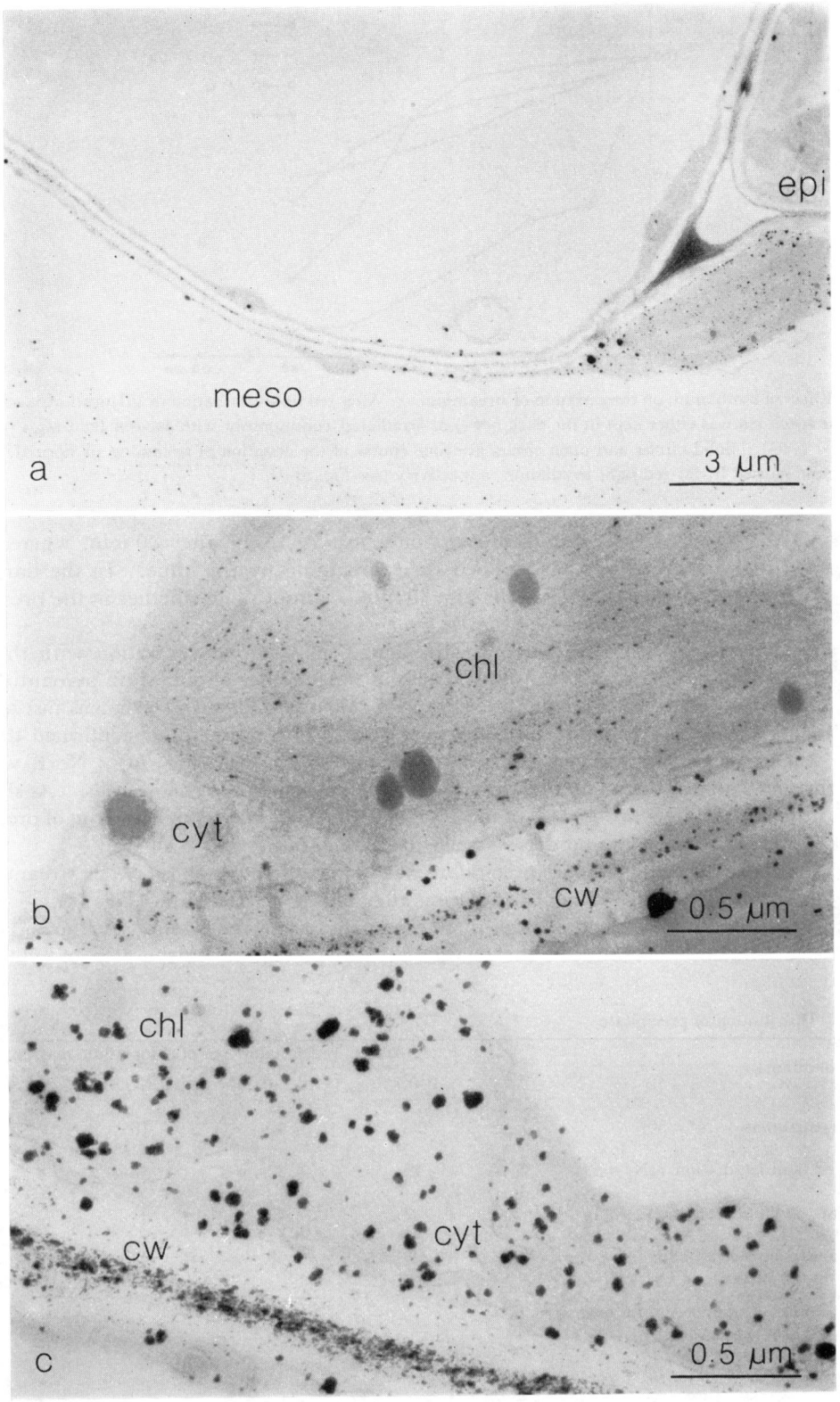

Fig. 8 Intracellular calcium deposits in cells fixed in the presence of potassium pyroantimonate. Sections were not stained. a: Cross section of cells kept in the dark for 12 - 18 h before the fixation. b: Cross section of mesophyll cells irradiated with red light. c: Cross section of mesophyll cells irradiated first with red light and then with far-red light. epi= epidermal cell, meso= mesophyll cell, chl= chloroplast, cw= cell wall, cyt= cytoplasmic matrix.

Table 3 Distribution of calcium precipitate

Conditions	Area of precipitate/Area of cytoplasmic matrix			
	0 - 0.1	0.1 - 0.2	0.2 - 0.3	0.3 - 0.4
Pretreatment (N = 50)	36	42	18	4
Red light irradiation (N = 53)	49	32	13	6
Far-red light irradiation (N = 54)	2	15	41	37
Far-red light irradiation in the presence of La^{3+} (N = 57)	28	58	12	2

N: The number of micrographs examined.

Values are given as the percentages to N.

four groups. The number of micrographs categorized in each group was expressed as the percentage to the total examined micrographs (N in Table 3). The relative area occupied by precipitate decreased when specimens were irradiated with red light, and markedly increased after subsequent far-red light irradiation. However, this increase was not observed in specimens irradiated with far-red light in 0.1 mM-$LaCl_3$-containing APW, in which the N_x/N_{total} ratio had been about 75 % just before the fixation.

Estimation of intracellular Ca^{2+} concentration regulating the streaming - Red and far-red lights seemed to control the streaming by changing Ca^{2+} concentration in the cytoplasm. To estimate intracellular Ca^{2+} concentration regulating the motility of the cytoplasm, I treated mesophyll cells with a solution containing various concentration of Ca^{2+} in the presence of A23187, which is known to make the cell membrane permeable to Ca^{2+} (Caswell & Pressman 1972, Reed & Lardy 1972, Wong et al. 1973). In mesophyll cells that had been kept in the dark for 12 h, the cytoplasmic particle, nucleus and chloroplasts stood still. Many of the chloroplasts were distributed almost randomly in the cortical layer of the cytoplasm (Fig. 9a). Only the mesophyll cells in the outermost layer were observed. First, I treated the cells with a test solution containing no $Ca(NO_3)_2$, of which Ca^{2+} concentration was supposed to be at less than 10^{-9} M. Agitation of all organelles occurred within 2 - 3 min, then directional saltation (Rebhun 1964) to the side wall was observed during the next 2 - 3 min. Rotational streaming along the side wall was established in all mesophyll cells 30 min after the

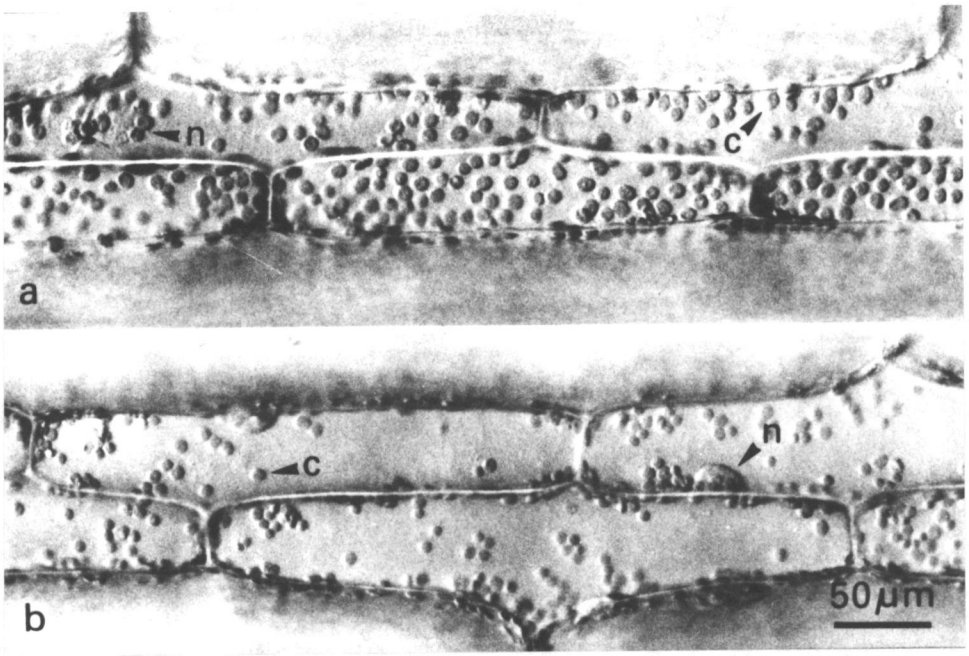

Fig. 9 Photomicrographs of Vallisneria mesophyll cells. Leaf segments are incised in the middle of the mesophyll cell layers. Rectangular shaped cells run parallel to the longitudinal axis of the leaf. a: Just after the pretreatment. The chloroplasts stand still and are distributed almost randomly in the cortical layer of the cytoplasm. b: The commencement of streaming. The cytoplasmic particles, nucleus and chloroplasts begin to stream along the side wall. c= chloroplast, n= nucleus.

application of the solution (Fig. 9b).

Next, to examine the effect of Ca^{2+} concentration on cessation of the streaming, I replaced the solution bathing the specimens, in which all mesophyll cells had exhibited streaming, with solutions containing various concentrations of Ca^{2+}. When they were treated with solutions of 10^{-8} M or 10^{-7} M Ca^{2+}, the streaming continued for 120 min in more than 80 % cells of the N_{total}. In a solution containing 10^{-6} M of Ca^{2+}, the streaming continued in the same number of cells as in the solutions of 10^{-8} M and 10^{-7} M of Ca^{2+} for the first 60 min, then gradually ceased. Solutions of 10^{-5} M and 10^{-4} M Ca^{2+} produced the cessation of streaming more rapidly, but the saltatory movement of the organelles, which was identified as the streaming according to our criterion, continued in about 20 % cells of the N_{total} (Fig. 10).

These results were further confirmed by the following observations. I transferred the specimens, which had been treated with the solution of 10^{-4} M Ca^{2+} and had no streaming cells, to solutions of various Ca^{2+} concentrations. 10 min after the application of solutions containing 10^{-8} M, 10^{-7} M and 10^{-6} M of Ca^{2+}, the streaming was induced in almost all cells. On the other hand, in solutions of 10^{-5} M and 10^{-4} M Ca^{2+}, only the transient saltatory movement of the cytoplasm was observed in a small number of cells (Fig. 11).

Unless the treatment exceeded 3 h, induction and cessation of the streaming were caused alternately with application of Ca^{2+} at 10^{-7} M and 10^{-4} M, respectively. Fig. 12 represents two

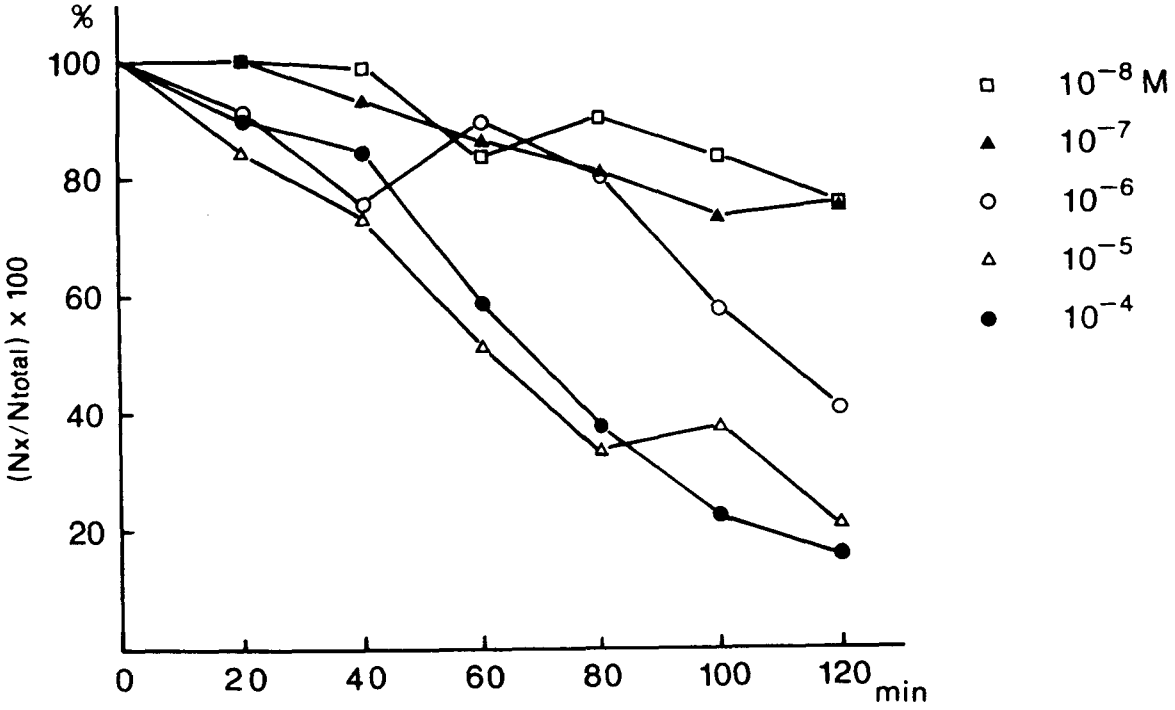

Fig. 10 Cessation of streaming and Ca^{2+} concentration. Specimens were treated with a solution containing Ca^{2+} at lower than 10^{-9} M for 30 min to induce streaming in all cells. Then the solution was replaced with ones containing various concentrations of Ca^{2+}. The N_x was counted at 20 min intervals, and the ratio of N_x to the N_{total} was plotted as percentage. 50 - 100 cells from five different leaf segments were examined for each test solution.

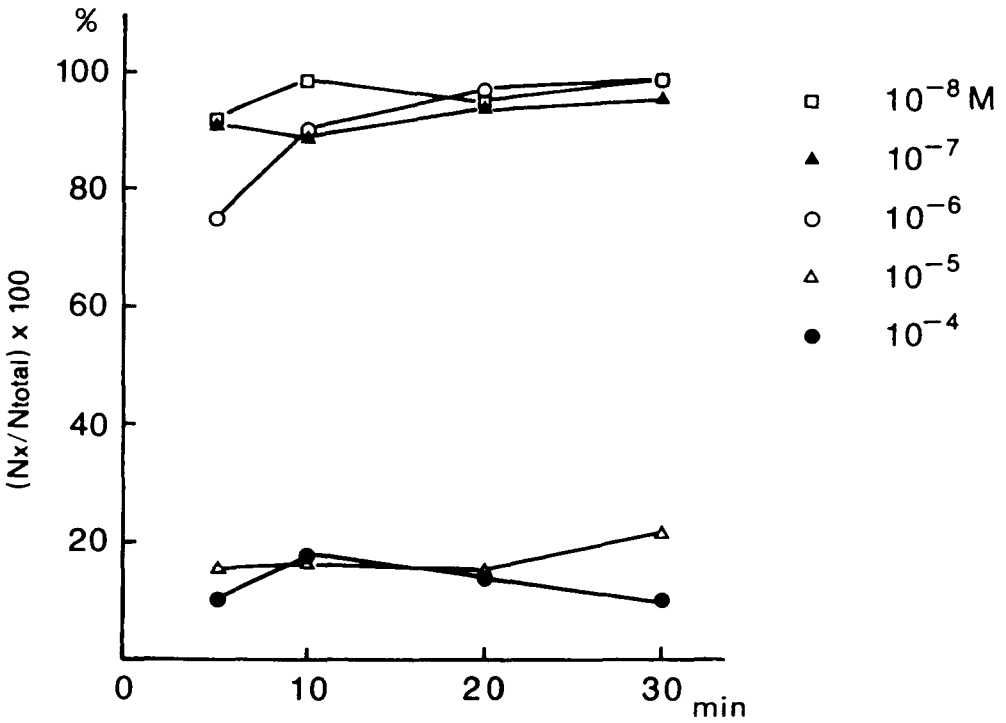

Fig. 11 Induction of streaming and Ca^{2+} concentration. Specimens, in which all cells had stopped their streaming in a solution of 10^{-4} M Ca^{2+}, were treated with solutions containing various concentrations of Ca^{2+}. The N_x was counted at 5 - 10 min intervals.

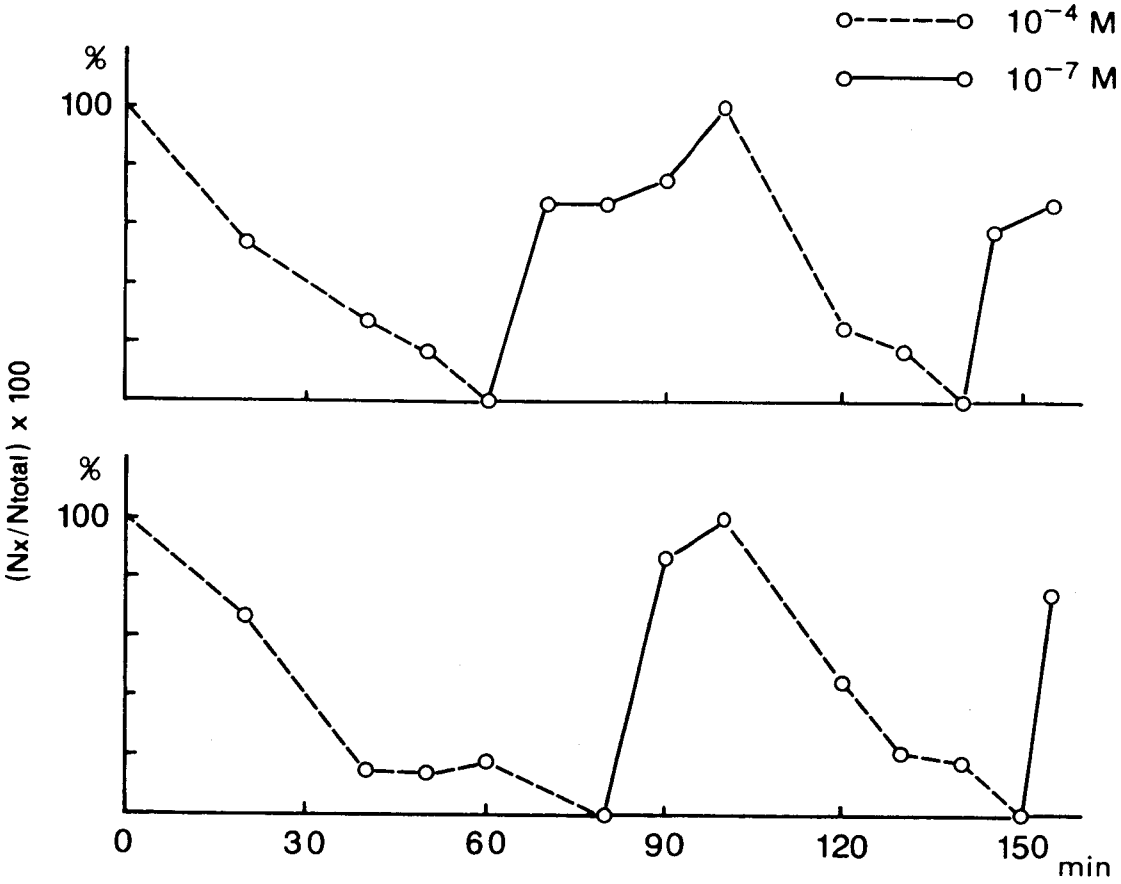

Fig. 12 Repetition of induction and cessation of streaming. First, streaming was induced in all cells by the application of a solution containing Ca^{2+} at lower than 10^{-9} M. Then the solution was replaced with one of 10^{-4} M Ca^{2+}. When the cells were treated with a solution of 10^{-7} M Ca^{2+} at the time streaming ceased, they resumed streaming. Unless the treatment exceeded 3 h, these induction and cessation cycles could be repeated. The N_{total} was 10 - 13 in a and 11 - 15 in b.

typical examples.

III: Ca^{2+} fluxes across the cell membrane

Effect of light irradiation on Ca^{2+} fluxes in protoplasts - The amount of calcium precipitate in the cytoplasm decreased when cells were irradiated with red light (Fig. 8 and Table 3). La^{3+} had no effect on the induction of streaming by red light irradiation. From these results, I have posturated a Ca^{2+} transport system which facilitates a Ca^{2+} movement from the cytoplasm to the outside of the cell, other than a La^{3+}-sensitive Ca^{2+}-channel. To ascertain this possibility, protoplasts were prepared from mesophyll cells. Protoplats are favorable for an ion flux assay, because they have no cell wall. Mesophyll cells were far more easily released than epidermal cells by the enzymatic digestion, and there was much difference in the size between the protoplast from mesophyll cells and that from epidermal cells (Fig. 13). The average diameter of protoplasts from mesophyll cells was 95 μm, and that from epidermal cells was 35 μm. These allowed me to get a homogeneous preparation of protoplasts from mesophyll cells. The contamination of epidermal cells was determined by counting the cell number, and was smaller than 0.5 %. The preparation also included about 1 % of non-spherical mesophyll cells which might be the results of the incomplete digestion.

30 min after the far-red light irradiation, protoplasts were irradiated with red light (650 nm, 0.4 W/m^2) for 5 min. The absorbance change of murexide showed an increase in Ca^{2+} concentration of the test solution, which meant an efflux of Ca^{2+}

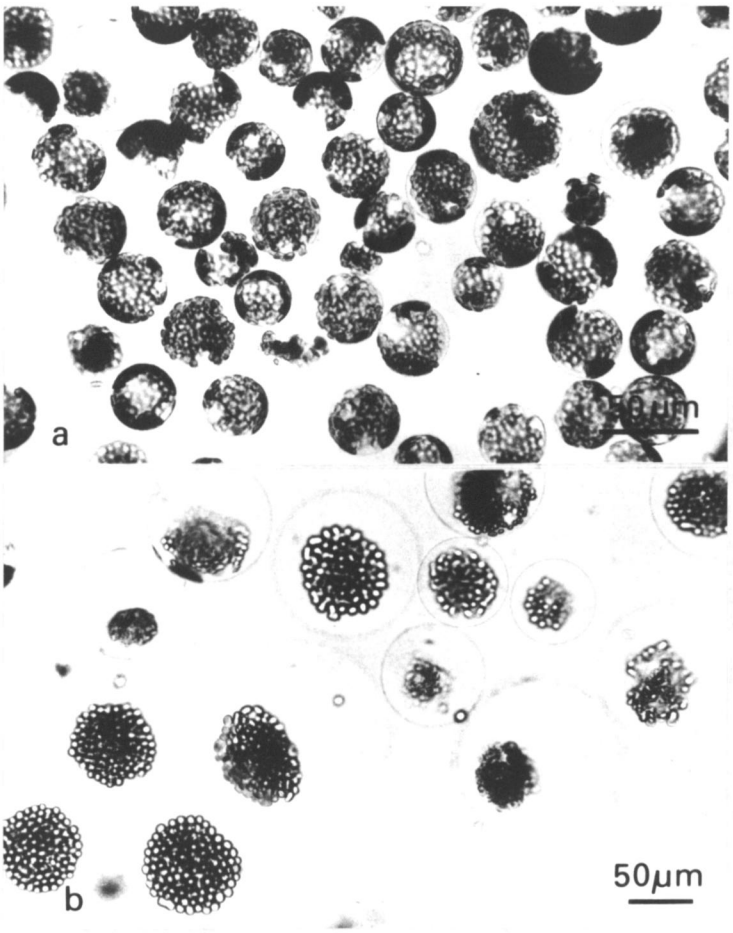

Fig. 13 Isolated <u>Vallisneria</u> protoplasts. a: Protoplasts prepared from epidermal cells. The average diameter of the protoplasts is 35 μm. b: Protoplasts from mesophyll cells used for Ca^{2+} flux assay. The average diameter of the protoplasts is 95 μm.

from the protoplasts (Fig. 14). Ca^{2+} concentration increased not only during the irradiation period, but also after the end of the red light irradiation. The reaction did not seem to be completed within 5 min of red light irradiation. The absorbance of murexide reached a constant value 3 min after the initiation of measurement. However, this increase in Ca^{2+} concentration was not induced by far-red light (730 nm, 0.3 W/m^2) irradiation for 10 min (Fig. 15). Then the same protoplasts were irradiated with red light for 10 min. Ca^{2+} concentration increased during the red light irradiation, and was constantly maintained for at least 2 min after the end of the irradiation.

Increased Ca^{2+} concentration by red light irradiation gradually decreased in the dark, indicating a slow reentry of Ca^{2+} into the protoplasts (Fig. 16a). 5 min after the end of red light irradiation, about half of Ca^{2+} released from the protoplasts was taken up again. In contrast, far-red light irradiation immediately after the red light irradiation produced a strikingly rapid decrease in Ca^{2+} concentration (Fig. 16b). Ca^{2+} concentration of the test solution decreased to about half in 2 min, and to almost the dark control level in 5 min of the far-red light irradiation.

<u>Effect of vanadate on Ca^{2+} fluxes</u> - There have been several reports on ATP-dependent Ca^{2+} transport across the cell membrane in plants cells (Gross & Marmé 1978, Dieter & Marmé 1980, Kubowicz et al. 1982). In this study, I examined the effect of vanadate which was used as an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase (O'Neal et al. 1979, Pick 1982) and other ATPases in

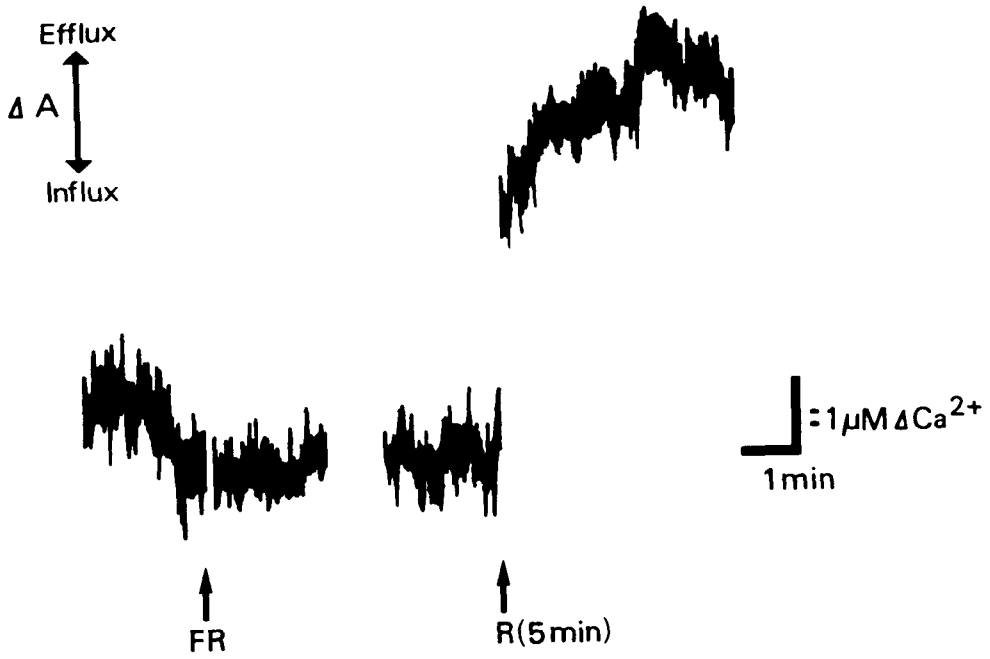

Fig. 14 Red light induced Ca^{2+} efflux from protoplasts. About 10^4 protoplasts were irradiated with far-red light (730 nm, 0.3 W/m^2) for 10 min (FR) immediately after the introduction into a measuring cuvette with the test solution. The absorbance of murexide reached a constant value 30 min after the end of the far-red light irradiation. Then the protoplasts were irradiated with red light (650 nm, 0.4 W/m^2) for 5 min (R(5 min)). The spectrophotometric measurement was started just after the end of the red light irradiation. The measurements were intermitted during actinic light irradiation. The ordinate shows an absorbance change of murexide at 544 nm; Efflux means a decrease in the absorbance and Influx means an increase in the absorbance. The reference beam was set at 500 nm.

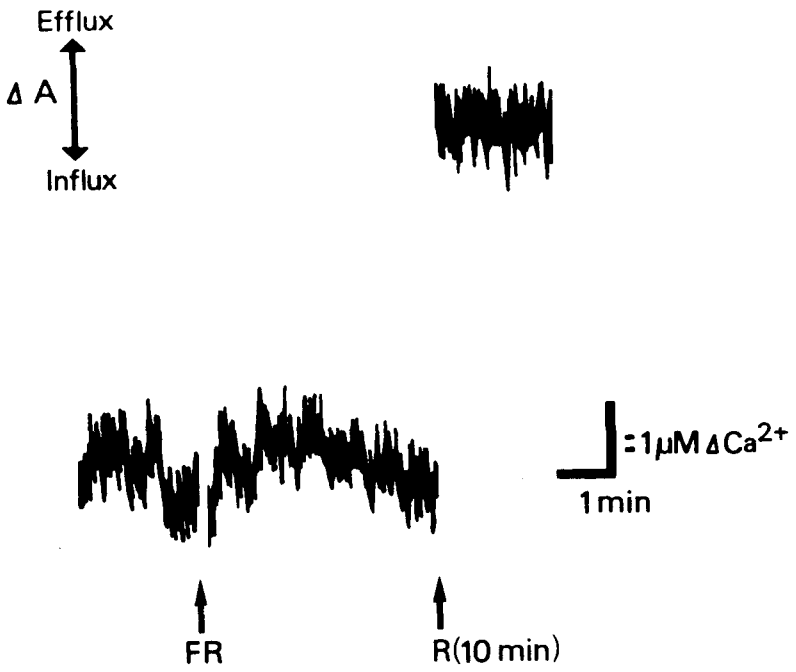

Fig. 15 Effects of red and far-red light irradiation on Ca^{2+} fluxes. 30 min after the far-red light irradiation as described in Fig. 14, protoplasts were irradiated again with far-red light (FR). After 4 min of the spectrophotometric measurement, the same protoplasts were irradiated with red light for 10 min (R(10 min)).

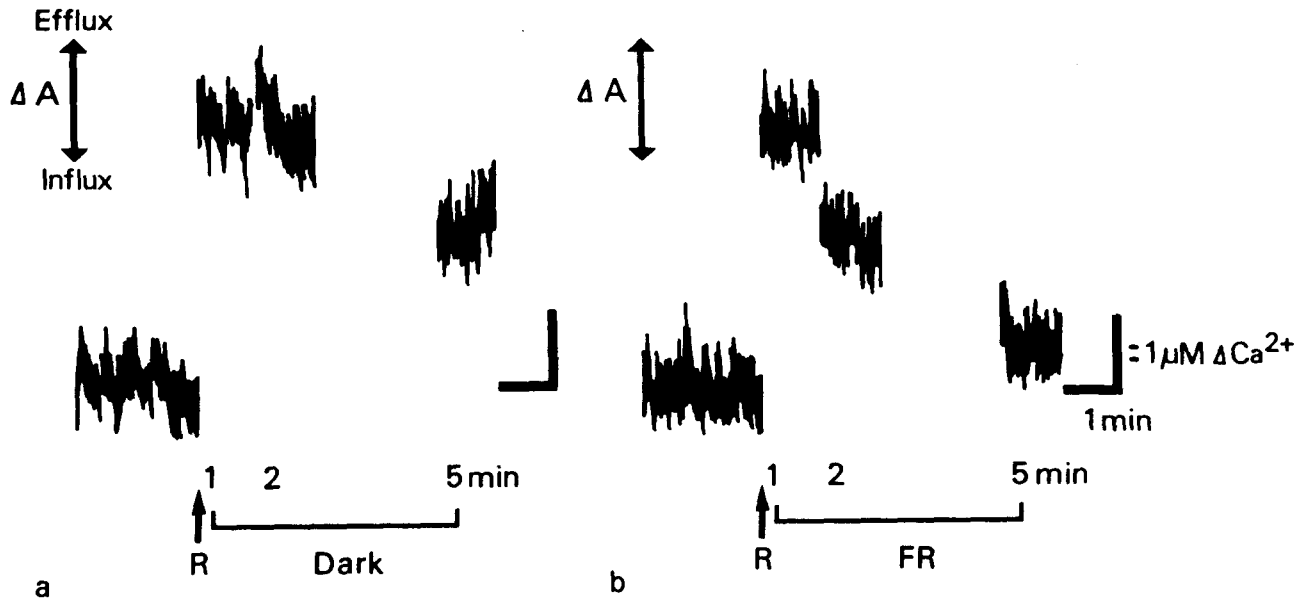

Fig. 16 Effect of far-red light irradiation on Ca^{2+} level after red light irradiation. After red light irradiation (R), protoplasts were either kept in the dark (a, Dark) or irradiated with far-red light (b, FR). The absorbance was measured 1, 2 and 5 min after the end of the red light irradiation. Each value is expressed in 1 min measurement.

the plant cell membrane (Bowman et al. 1978, Willsky 1979, O'Neill & Spanswick 1984). The first red light irradiation brought about an increase in Ca^{2+} concentration in the same way as described above (Fig. 17, R). After the subsequent far-red light irradiation (FR), the protoplasts were treated with sodium orthovanadate at 10 μM for 30 min in the dark. The next red light irradiation (R, +10 μM vanadate) had no effect on Ca^{2+} concentration of the test solution. Thus the red-light induced Ca^{2+} efflux from the protoplasts was inhibited in the presence of vanadate. This also indicated that red light induced Ca^{2+} efflux in the absence of vanadate was not due to unspecific dissociation of Ca^{+2} from the cell membrane.

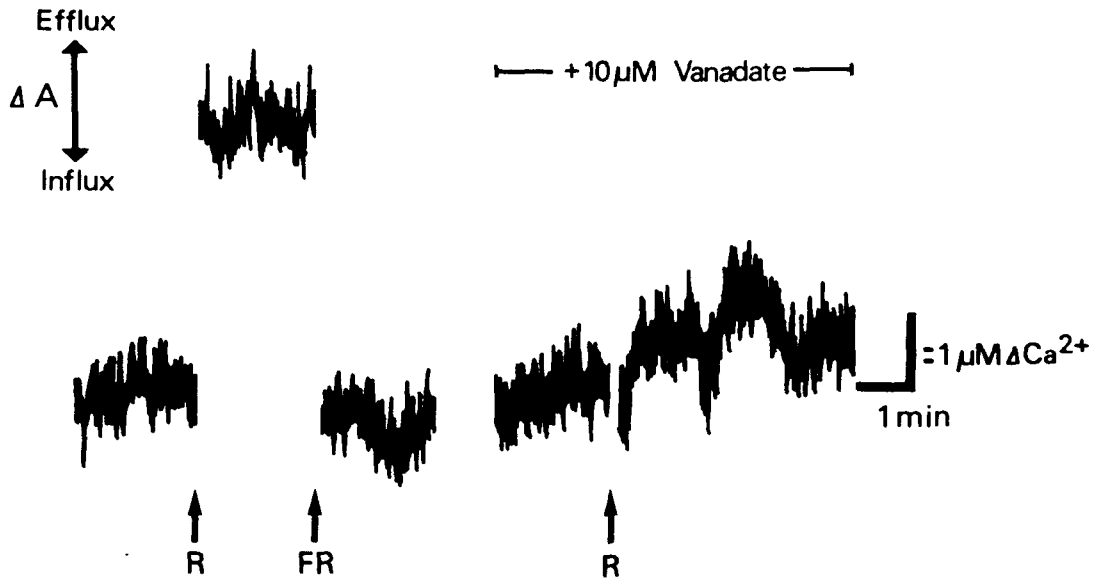

Fig. 17 Effect of vanadate on Ca^{2+} efflux. The viability of protoplasts was confirmed with Ca^{2+} efflux induced by the first red light irradiation (R). Then the protoplasts were irradiated with far-red light (FR), and sodium orthovanadate was added to the test solution at final concentration 10 μM. 30 min after the application, the protoplasts were irradiated again with red light (R).

Discussion

Effect of light irradiation on the streaming - The following three points indicate that phytochrome is involved in the reaction as a light-absorbing pigment. First, light of 650 nm was most effective to induce the streaming (Fig. 2 and 3). This wavelength corresponds to the absorption peak in P_r. Second, the inhibitory effect of far-red light did not decrease significantly at wavelengths longer than 730 nm (Fig. 6). Continuous light irradiation is known to produce a photostationary state in the phytochrome. As P_{fr} in the total phytochrome decreases with increase in the far-red light in the incident light, the ratio of P_{fr} to the total phytochrome reaches a minimum when cells are irradiated continuously with light of over 700 nm (Butler et al. 1963, Hartmann 1966). Therefore it is reasonable to assume that the effect of far-red light did not decrease in the region from 700 to 800 nm. I can also assume that P_{fr}, a physiologically active form of phytochrome, is involved in the induction and maintenance of streaming here (cf. Kraml & Schäfer 1983). Third, the photoreversibility was found in the pulse irradiation tests (Table 2). Although an inhibitory effect of far-red light on the streaming was produced in 1 min irradiation, the effect was almost completely reversed by the subsequent red light irradiation. And the same is true for the next, second red and far-red light irradiation. This strongly suggests that reversible phototransformation of the pigment is integrated into the regulatory mechanism of streaming.

No movement of chloroplasts was observed in the first 2.5 x 10^2 s of irradiation with red light at any intensity (Fig. 4). I consider this an apparent latent period between the perception of light and the start of streaming in the most sensitive cell. A careful examination of this latent period will provide the necessary information on the initiation of the reaction.

Seitz (1967) determined the action spectrum for photodinesis in V. spiralis epidermal cells. He found that blue light was most effective and proposed riboflavine as the main photoreceptive pigment. In this study, cells were irradiated at considerably low intensity, less than 1/10 of that in the case of Seitz, and continuous red light irradiation (1.9 W/m^2) fully induced streaming in 30 min. Red light at this intensity did not induce streaming in epidermal cells and blue light (450 nm) at a higher intensity (26 W/m^2) did not show significant effect on mesophyll cells (data not shown). Hitherto, I do not know whether these discrepancies can be attributed to the difference between epidermal and mesophyll cells or between V. spiralis and V. gigantea. If the former is the case, it is interesting that two adjacent cell types have two different kinds of pigment to control their photodinesis. Photoreceptive pigment(s) in V. gigantea epidermal and mesophyll cells must be investigated more precisely.

Distribution of calcium precipitate - Increase and decrease in Ca^{2+} concentration in the cytoplasm were visually demonstrated using a specific precipitant of calcium, potassium

pyroantimonate. This reagent penetrates the biological membrane and forms precipitate with calcium (Caswell 1979, Wick & Hepler 1980). As it reacts with some cations other than Ca^{2+}, we have confirmed the presence of calcium in the precipitate by X-ray microanalysis (Takagi & Nagai 1983). There is a considerable difference between the amount of precipitate formed in mobile cytoplasm and that formed in immobile cytoplasm under far-red light irradiation (Fig. 8b and Fig. 8c, Table 3). These observations are consistent with our previous conclusion that streaming is induced when Ca^{2+} concentration in the cytoplasm decreases and is inhibited when it increases (Takagi & Nagai 1983).

Kuroda & Kuroda (1982) fixed a dumbbell-shaped plasmodium of Physarum polycepharum exhibiting active shuttle streaming in the presence of antimony. They examined the relation between the number of precipitate in the cytoplasm and the contractility of the plasmodium. In this study, the relative area of precipitate was examined instead of the number of precipitate. I thought the relative area might better reflect the Ca^{2+} concentration than the number of precipitate, because the precipitate comprises a wide variety of sizes.

The inhibitory effect of far-red light on the streaming did not appear in La^{3+}-containing APW (Fig. 7). La^{3+} inhibits wound-healing motility in Ernodesmis (La Claire 1982) and light-induced germination of spores of Onoclea (Wayne & Hepler 1984). Ca^{2+} is supposed to be involved in both reaction. We have already reported that cessation of streaming caused by far-red light

irradiation was attributed to the increase in Ca^{2+} concentration in the cytoplasm (Takagi & Nagai 1983). Table 3 indicates that La^{3+} blocked Ca^{2+} influx through the cell membrane and slowed down the cessation of streaming. Ca^{2+} transported into the cytoplasm from outside during far-red light irradiation must play a crucial role in the cessation of streaming.

Red and far-red lights may possibly regulate the streaming by changing Ca^{2+} concentration in the cytoplasm. There have been some investigations on light-controlled Ca^{2+} transport systems in plant cells. Calcium accumulation in Mougeotia cells is enhanced by red light irradiation and this effect is cancelled by subsequent far-red light irradiation (Dreyer & Weisenseel 1979). Hale & Roux (1980) reported that Ca^{2+} efflux from etiolated Avena coleoptiles and their protoplasts was stimulated by red light irradiation, and that subsequent far-red light irradiation reversed the effect. They also examined the characteristics of Ca^{2+} transport in isolated mitochondrial fraction, and proposed that an increase in Ca^{2+} concentration in the cytoplasm is caused by P_{fr}-promoted Ca^{2+} efflux from mitochondria (Roux et al. 1981)

Estimation of Ca^{2+} concentration - Several methods which increase the permeability of the cell membrane have been developed in plant cells to control chemical composition of the cytoplasm. Shimmen & Tazawa permeabilized the plasmalemma of Nitella internodal cell by inducing plasmolysis at low temperature in the presence of EGTA (1983a), or by applying an electric stimulus in Ca^{2+}-lacking APW (1983b). They examined the effects of chemicals

such as ATP and cytochalasin B on the cytoplasmic streaming in the permeabilized cell. Yoshimoto & Kamiya (1984) prepared saponin models of Physarum polycepharum plasmodium strands to study how Ca^{2+} and ATP regulate the tension generation. The cytoplasmic streaming in Vallisneria mesophyll cells is easily induced by chemical application or by physical injury (cf. Kamiya 1959, Takagi & Nagai 1983). To avoid any side effects caused by drastic treatments with a hypertonic solution, detergent and so on, the specimen was treated with the test solution containing a divalent ionophore A23187 in this study. This drug seemed to be suitable for the long-lasting treatment of cells. In target cells of Funaria hygrometrica protonema, Saunders & Hepler (1982) induced the initial cell division which led to bud formation. They treated the plant over 24 h with Ca^{2+}-containing medium in the presence of A23187. A23187 also induced the rotational movement of chloroplast in Mougeotia in Ca^{2+}-containing medium (Serlin & Roux 1984). The reaction started 90 min after the application and almost finished in the following 30 min without detriment to the viability of cells. I used the drug successfully by cutting open the mesophyll cell layers and exposing the cells directly to the test solution. The cell wall of the mesophyll cell is thin enough to allow the drug to permeate and exert its effect on the cell membrane. Application of the test solution without the drug had no effect on the induction and cessation of streaming (data not shown).

Volume of each test solution relative to the volume of a specimen was larger than 150. Streaming was induced within 30

min by treatment with a test solution containing no $Ca(NO_3)_2$ in all mesophyll cells in the specimen just after the pretreatment. In the repetition test, Fig. 12 shows that the second induction by 10^{-7} M-Ca^{2+} treatment takes almost the same time to reach 100 % N_x/N_{total}. The same was observed in the first and second cessation caused by 10^{-4} M-Ca^{2+} treatment. Therefore I considered intracellular Ca^{2+} concentration of the specimen to be fully controlled with the test solution.

The results indicate that Ca^{2+} at 10^{-8} M - 10^{-6} M activates the system for the motive force generation, and inhibits it at concentrations higher than 10^{-5} M. The situation is the same in Characean cells (Hayama et al. 1979, Tominaga & Tazawa 1981, Williamson & Ashley 1982), but different from that in Physarum (Yoshimoto & Kamiya 1984) and Acetabularia (Fukui & Nagai 1985). The chloroplast movement in the green alga Mougeotia (Dreyer & Weisenseel 1979, Serlin & Roux 1984) and wound-healing motility in the marine alga Ernodesmis (La Claire 1982, 1983) require an influx of exogenous Ca^{2+}, although the intracellular Ca^{2+} levels suitable for the movement have not been determined. The difference in the mode of Ca^{2+} regulation may be attributed to the difference in controlling factor(s) of the mechanism for motive force generation in each plant.

Light-controlled Ca^{2+} fluxes - Ca^{2+} efflux from protoplasts was induced by red light irradiation, but not by far-red light irradiation (Fig. 15). Far-red light irradiation immediately after the red light irradiation rapidly decreased Ca^{2+}

concentration of the test solution (Fig. 16b). These observations indicate that light irradiation affects Ca^{2+} movement across the cell membrane, and that phytochrome is involved as the photoreceptive pigment. The latter statement is supported by another evidence that far-red light irradiation, which might transform P_{fr} to P_r in the protoplasts, 30 min before the Ca^{2+}-flux assay has assured consistent results of the experiments. By measuring absorbance changes of murexide, Hale & Roux (1979) found that Ca^{2+} efflux was induced by red light irradiation in Avena protoplasts from apical tips of etiolated coleoptile. However, this Ca^{2+} efflux was not observed in protoplasts from greened leaf mesophyll cells, in which phytochrome was not detectable by spectrophotometry. The phytochrome content in protoplasts from Vallisneria mesophyll cells must be estimated by immunological method or by spectrophotometry.

Das & Sopory (1985) observed that $^{45}Ca^{2+}$ uptake in protoplasts from etiolated Zea leaf was accelerated by red light irradiation, and that the effect was reversed by far-red light irradiation immediately after the red light irradiation. Red light stimulated Ca^{2+} uptake in plant cells were also demonstrated in Mougeotia by macro-autoradiography (Dreyer & Weisenseel 1979), and in Onoclea spores by atomic absorption analysis (Wayne & Hepler 1985). An apparent direction of Ca^{2+} movement across the cell membrane in those cases (influx) is opposed to that in the case of Vallisneria (efflux). I do not know whether the difference in the direction of Ca^{2+} movement can

be attributed to the heterogeneity in cell materials (species, etiolated or not, ect.) or to the experimental procedures. Examining effects of light irradiation on Ca^{2+} influx in Vallisneria will provide a decisive answer to this question.

Red light induced Ca^{2+} efflux was not observed in the presence of 10 μM vanadate (Fig. 17). Gross & Marmé (1978) isolated membrane vesicles from several kinds of plant cells, and demonstrated energy-dependent Ca^{2+} uptake into those vesicles against a concentration gradient. They interpreted the observations as an active Ca^{2+} transport from the cytoplasm to the outside of the cell. However, Ca^{2+} uptake activity of the vesicles was insensitive to vanadate (Dieter & Marmé 1980), which inhibited sarcoplasmic reticulum Ca^{2+}-ATPase at 10 μM order (O'Neal et al. 1979, Pick 1982) and other ATPases in the plant cell membrane (Bowman et al. 1978, Willsky 1979, O'Neill & Spanswick 1984). Although I applied the drug exogenously, Cantley et al. (1978) reported that vanadate was transported into the red cell and inhibited the Na^{+},K^{+}-ATPase from the cytoplasmic side. Therefore the inhibitory effect of vanadate on Ca^{2+} efflux in Vallisneria does not seem to be due to nonspecific damage on the cell membrane.

Induction of streaming by red light irradiation in Vallisneria mesophyll cells was inhibited by 60 % in the presence of 10 μM vanadate (data not shown). Shimmen & Tazawa (1982) observed that cytoplasmic streaming in Nitellopsis took place even when the cytoplasm was treated with 1 mM vanadate, and proposed that the vanadate-insensitive actomyosin system was

concerned with the motive force generation. In Vallisneria, it has almost been revealed that the streaming is driven by the actomyosin system (Ohsuka & Inoue 1979, Ishigami & Nagai 1980, Yamaguchi & Nagai 1981, Takagi & Nagai 1983). Inhibition of both Ca^{2+} efflux and streaming by vanadate indicates that Ca^{2+} transport system in the cell membrane, in which phytochrome takes part as the photoreceptor, regulates the cytoplasmic streaming in Vallisneria mesophyll cells. Phytochrome most plausively regulates Ca^{2+}-translocating ATPase in the cell membrane. Isolation of membrane vesicles from Vallisneria mesophyll cells and chracterization of the Ca^{2+} transport activity will reveal the phytochrome-mediated regulatory mechanism of Ca^{2+}-translocating ATPase.

Other than Ca^{2+} transport system in the cell membrane, cell organelles such as mitochondria (Roux et al. 1981), chloroplasts (Muto et al. 1982) and endoplasmic reticulum (Hepler 1980, Wick & Hepler 1980) must be involved in the controlling mechanism of intracellular Ca^{2+} concentration. Their spatial and temporal implication remains to be investigated.

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