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Germination and Photo-Induction of Polarity in the Spherical Cells Regenerated from Protoplasm Fragments of *Boergesenia forbesii*

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The processes of rounding (spheration) and cell wall formation of extracellular protoplasm fragments of *Boergesenia forbesii* were examined. This spheration depended on the presence of Ca^{2+} in the medium, and was accelerated exponentially with increasing temperature up to 35 C. At 25 C the regenerated cell wall could be detected within 3 hr, after the release of protoplasm into Provasoli's ES-medium.

Germination, the development of a protrusion from the round cell, had an optimal temperature of 30 C and was interrupted at temperatures below 17 C. The germination was promoted by red-light irradiation (a maximum around 625 nm). This promotion depended on both irradiation time and light intensity, and was diminished by DCMU. Therefore, it was concluded that this promotion was caused predominantly through photosynthetic activity.

The rhizoidal protrusion developed on the shaded side of a unilaterally irradiated round cell. Blue light was most effective in inducing cell polarity with light of wavelengths longer than 600 nm being ineffective. This wavelength-dependency was similar to that observed for polarity induction of *Fucus* eggs or in phototropism of *Avena* coleoptiles.

Boergesenia forbesii (Harvey) Feldmann (Chlorophyceae, Siphonocladales) is a gigantic unicellular marine alga whose cell growth and physiological properties had not yet been well studied. Through cultural studies of this alga (Enomoto and Hirose, 1972), it was found that osmotic or mechanical stress on the cell induced rapid aplanospore formation. Furthermore, it was observed that extracellular protoplasm fragments could easily develop into new cells in sea water.

The technical achievement of *in vitro* preparation of many homogeneous cells and their growth in artificial medium, made it feasible to investigate the cell growth characteristics in detail. The principal objective of this study was to determine the method of *in vitro* preparation of protoplasts and new cells, and major characteristics of this regeneration process. Growth and development of newly-formed cells was profoundly affected by the conditions of light irradiation, as well as temperature. Their effects on germination, the first step of the cell growth, were investigated.

Materials and Methods

Culture

Boergesenia plant was originally collected at Amami-ōshima Island, Japan (Enomoto and Hirose, 1972). The alga has been cultured through aplanospore formation, with removal of contaminating microorganisms. The culture solution was Provasoli's ES-medium (ESP-medium; Provasoli, 1966), prepared from sea water filtered through sterilizing film (Toyo-Roshi 85SB) and autoclaved at 120 C for 20 min. The alga was grown at 25 C under a 16 hr light and 8 hr dark regime. The illumination was 2–3 $W \cdot m^{-2}$ with white fluorescent tubes (Hitachi FLR-80H-W/A and Toshiba Plantlux FL40S·BR/NL).

Preparation of spherical cells from protoplasm fragments

The alga was cultured for 2 months, at which time it was about 15 mm tall. The plant was immersed for 10 min in sea water to which 0.6 M mannitol was added to induce plasmolysis, then transferred to ESP-medium, and scissored into pieces to release the protoplasm fragments into the medium. The large number of fragments thus obtained were maintained at 25 C for 4 hr in the dark. During this period, the fragments of indefinite shape became spherical and generated cell walls, which were strong enough to resist the mechanical stress of sieving. These newly-formed, spherical cells were suspended in ESP-medium and filtered through nylon meshes to obtain a population of cells homogeneous in size.

Germination test

Twenty spherical cells were sown in a polyethylene dish 3 cm in diameter, containing 2 m*l* ESP-medium. Four dishes were set on moist filter paper in a 9 cm Petri dish. The development of a rhizoidal protrusion from the spherical cell (germination) was observed or photographically recorded under a low-power microscope. The germination rate was determined with 80 cells incubated in four dishes each containing 20 cells.

In a preliminary experiment, the spherical cells were classified by nylon-mesh filtration into five groups according to their diameter (40–69 μm, 69–106 μm, 106–130 μm, 130–174 μm and 174–200 μm) and their germination processes were examined at 25 C in the dark. The highest rate of germination was observed in the 106–130 μm group. Therefore, this group of cells was selected as material for the following experiments.

In the experiments on the light induction of polarity, the cells were sown on a thin layer of 0.6% agar in a rectilinear glass chamber ($2.9 \times 3.2 \times 2.6$ cm^3) containing 3 m*l* of ESP-medium. They were incubated at 28 C and illuminated unilaterally through the side wall of the chamber.

Light sources

Fluorescent tubes (Toshiba FL20SW) were used as the white light source, the red light source was colored fluorescent tubes (Mitsubishi FL20R-F) equipped with a red plastic filter (Mitsubishi-Jushi) 2 mm thick, and far-red light was supplied by incandes-

cent lamps (Toshiba 125 W) filtered through a 10 cm water layer and 2 mm-thick red and blue plastic filters. This combination of plastic filters did not transmit light of wavelengths shorter than 660 nm, although the transmittance at 680 nm was 27%. Therefore, a slight amount of red light was included in the "far-red" light used in this experiment. The wavelength dependency of germination was examined using a monochromator equipped with a xenon lamp and interference filters (High Energy Monochromator FX-28, Tokiwa-Koden). During examination of the wavelength dependency of polarity induction, monochromatic light was obtained using interference filters (Nippon Shinku-Kogaku) and a 3 cm water layer with a low voltage incandescent lamp in a microscope-light source (Nippon Kogaku).

Chemicals

Indoleacetic acid (IAA), naphthaleneacetic acid (NAA), gibberellic acid (GA_3), kinetin, sodium azide (NaN_3), cycloheximide and Triton X-100 were obtained from Wako Chemical Industries, Ltd., Japan. (±)-*cis*, *trans*-/all *trans*-Abscisic acid (ABA) were from Sigma Chemical Co., USA. 3-(3′,4′-Dichlorophenyl)-1,1-dimethylurea (DCMU) was from K & K Laboratories, USA. All reagents except DCMU were dissolved in the sterilized ESP-medium.

Results

Regeneration of protoplasm fragments to spherical cells

When small pieces of protoplasm were extracted from the *Boergesenia* plant into sea water as described in the Materials and Methods section, the irregularly shaped pieces of various sizes immediately began to change their forms so as to reduce their surface area, and finally became spherical (Fig. 1 A-D). This process of deformation of protoplasm *in vitro*, which was designated "spheration" in this paper, was the first step toward regeneration of new cells. The protoplasm balls smaller than about 30 μm in diameter, experienced difficulty in developing further under the present culture conditions, though most of them were multinucleate. Therefore, the spheration process was examined with the larger fragments 50–200 μm in length.

Deformation of fragments was highly accelerated by raising the temperature of the medium. At 20 C, it took about 1 hr to form round protoplasts under white fluorescent illumination of about 3 $W \cdot m^{-2}$. As shown in Fig. 2, it took only 3 min at 35 C, the

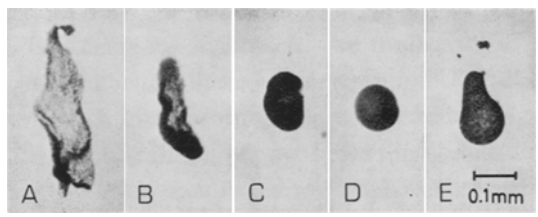

Fig. 1. Spheration process for the protoplasm fragment (A–D) and germination of the newly-formed cell (E).

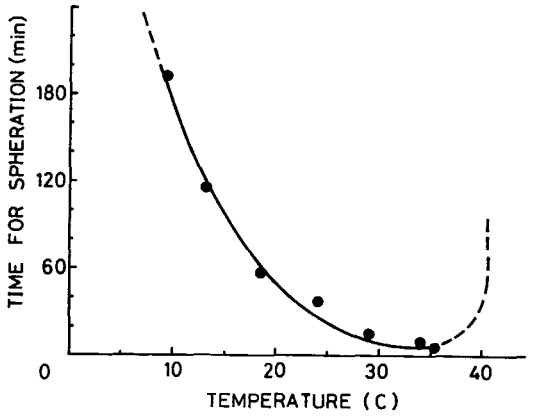

Fig. 2. Temperature dependency for the spheration of the protoplasm fragments. Ordinate is the time required for half of the fragments to become round at various temperatures. The values were determined with a lot of 240 fragments.

optimal temperature. Spheration occurred slowly both at high temperatures near 40 C and also at low temperatures around 5 C, and was not complete even after 24 hr. Consequently, the rate of spheration increased exponentially with temperature in the range of 10–35 C.

As shown in Table 1, the spheration and regeneration occurred normally in van't Hoff's artificial sea water. However, if calcium, but not the other ions, was eliminated

Table 1. Necessity of the presence of calcium in medium for spheration of *Boergesenia* protoplasm fragments

Medium		Spheration rate (%)[1]
van't Hoff's artificial sea water		100
〃	$-Mg^{2+}$ [2]	100
〃	$-Ca^{2+}$ [2]	0
0.6 M mannitol +0.45 M sucrose		0
〃 + 〃	+0.01 M Ca^{2+}	100

[1] Spheration rate was counted with a lot of 80 fragments after a 24 hr-incubation at 25 C.

[2] An equimolar concentration of Na^+ was supplemented.

from the medium, spheration could not proceed. On the other hand, the spheration occurred successfully in 10^{-2} M $CaCl_2$ solution containing 0.6 M mannitol and 0.46 M sucrose as osmotic agents. (This medium was osmotically equivalent to the sea water used in this experiment).

The round, multinucleate protoplasts could not develop further in the $CaCl_2$ medium, however, in ESP-medium or sea water, they generated cell walls rapidly and developed into new cells. The regeneration of cell walls could first be detected within about 3 hr by microscopic observation of the protoplasts stained with 0.07% ruthenium red. Then after about 6 hr, they could be observed either by fluorescence microscopy of the calcofluor-stained cells or by polarizing microscopy of the birefringent walls.

Progress in the regeneration of the cell wall could be estimated by the response of protoplasts to Triton X-100, a non-ionic surfactant. When the newly-prepared

fragments were treated with 0.05% Triton X-100 dissolved in ESP-medium, all fragments disintegrated within 8 min. As for the protoplasts kept in the dark at 25 C for 3 hr, the rate of disintegration caused by 0.05% Triton X-100 was less than 30%.

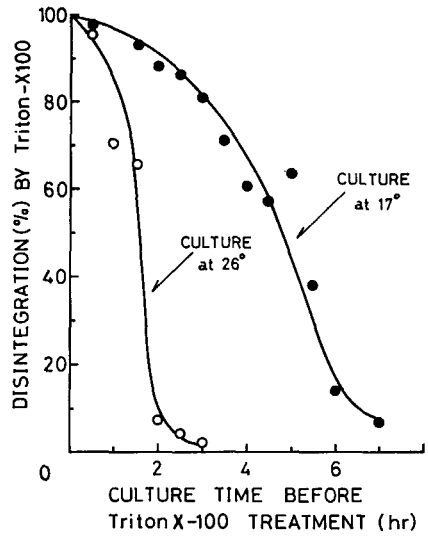

Fig. 3. Disintegration of the protoplasts by 0.05% Triton X-100. The rate of disintegration (ordinate) was measured with 60 protoplasts treated with 0.05% Triton X-100 (in ESP-medium) for 8 min. Each lot of 60 protoplasts had been cultured at 17 C or 26 C.

This increased resistance of protoplasts to Triton X-100 appears to reflect the strength of the regenerated cell walls. In the experiment shown in Fig. 3, the change in the sensitivity to Triton X-100 was measured with protoplasts cultured at different temperatures. A cell wall strong enough to resist Triton X-100 appeared to be formed in 3 hr at 26 C, while at 17 C it took 7 hr. This indicated that the rate of cell wall formation was also dependent on temperature.

Germination

The initial step of growth in the newly-formed cells was the formation of a rhizoidal protrusion or germination (Fig. 1 E). Irradiation by light was not necessary for this germination process. On the other hand, temperature had a great influence on germination. In the experiment shown in Fig. 4, germination rates were determined after 6, 9 or 12 days of incubation in the dark at various temperatures. The cells could germinate only within the temperature range of 20 to 33 C, but even after resting for one month at 15 C, they did not lose the ability to germinate.

The germination of this material, which was not accompanied by cell division, appeared simple compared with other known multicellular plant germination phenomena. In order to gather insight into the understanding of the protrusion process, the effects of metabolic inhibitors and growth regulators were examined. The results are summarized in Table 2. The strong inhibition by cycloheximide, and a significant inhibition by sodium azide indicated that both protein synthesis and respiration were essentially involved in this protrusion process. The exogenous

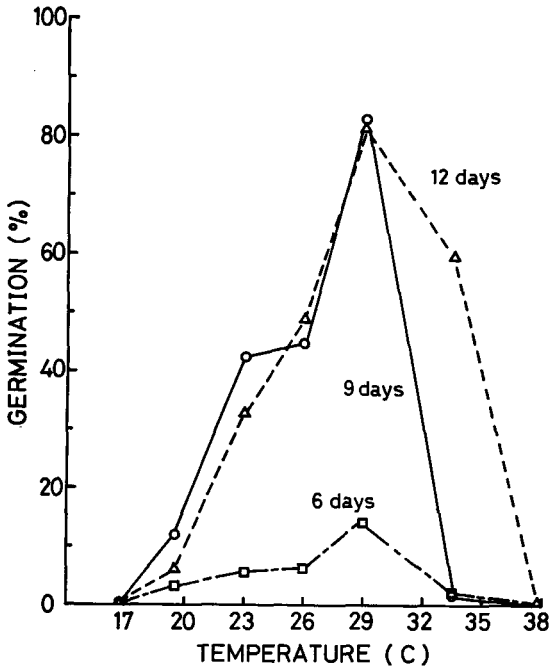

Fig. 4. Temperature dependency of the germination. The germination rates were determined after 6-day (□), 9-day (○) or 12-day incubation (△) in the dark. The germination rate was determined with a lot of 80 cells.

Table 2. Effects of some metabolic inhibitors and growth regulators on the germination of new cells

Reagent[1)]	Concentration (M)	Germination (%)[2)]
Control	–	69± 6[3)]
Cycloheximide	10^{-4}	0
NaN_3	10^{-3}	30± 8
〃	10^{-4}	47± 6
IAA	10^{-4}	52± 5
〃	10^{-5}	50± 1
NAA	10^{-4}	25± 1
GA_3	10^{-4}	88± 3
〃	10^{-5}	88±10
Kinetin	5×10^{-6}	95± 4
〃	5×10^{-4}	89± 4
ABA	10^{-4}	41± 2
〃	10^{-5}	37± 3

1) The pH value of the control (ESP-medium) was 8.2, and the pH change caused by dissolving the test reagents was less than 0.1.
2) After preincubation (17 C, 3 days) in ESP-medium, the spherical cells were incubated in the media containing the reagents for 6 days at 25 C in the dark. Germination rate was measured with 80 cells, cultured in four dishes each containing 20 cells.
3) Standard error.

application of plant growth regulators at the concentrations indicated in Table 2 affected the germination, with ABA, IAA and NAA inhibiting germination and GA_3 and kinetin promoting the germination.

Promotive effect of red light on germination

Though the cells could germinate in the dark as mentioned above, it was found that light irradiation still had a profound influence on the germination process. Fig. 5

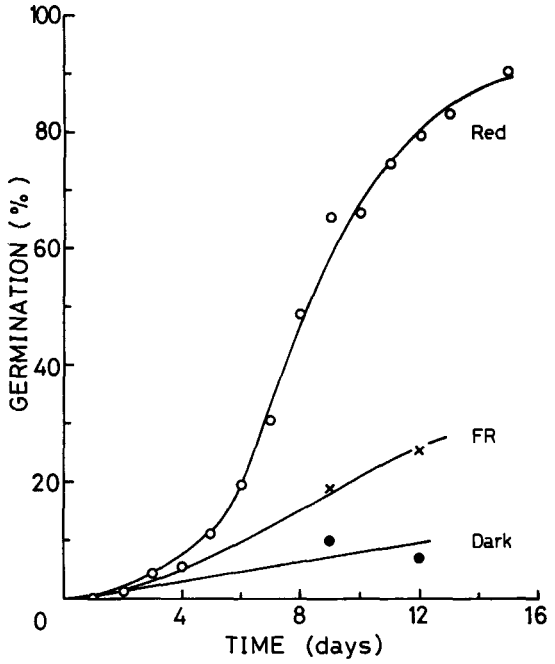

Fig. 5. Effect of red and far-red light upon the germination time course. After preincubation for 3 days at 17 C in the dark, cells were irradiated continuously with red (0.8 $W \cdot m^{-2}$, ○) or far-red light (FR, 1.0 $W \cdot m^{-2}$, ×) at 20 C. The closed circle (●) indicates the control in the dark. The germination rates were determined with lots of 240–400 cells.

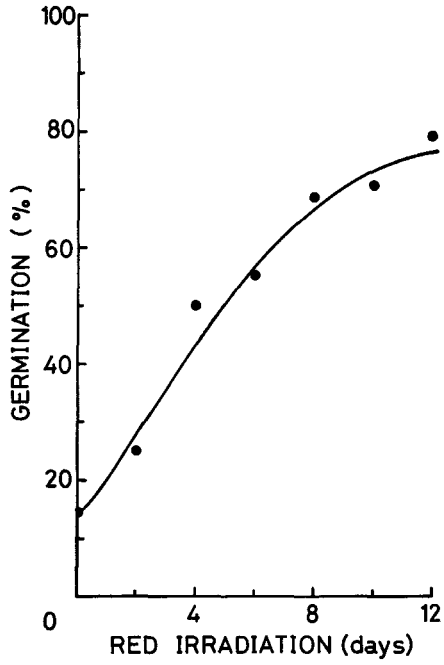

Fig. 6. Relationship between the duration of red-light irradiation and the germination rate. After preincubation for 3 days at 17 C in the dark, cells were irradiated with red light (0.8 $W \cdot m^{-2}$) for the periods shown on the abscissa, and then kept in the dark at 20 C. The germination rates were determined 12 days after the start of irradiation, with lots of 160 cells.

Table 3. The relationship between red-light intensity and the germination rate

Light intensity ($W \cdot m^{-2}$)[1)]	Germination (%)
0.73	76± 6
0.29	47± 4
0.15	52±10
0.03	23± 4
dark	11± 4

1) Continuous illumination with red light during 8-day incubation at 20 C. The light intensity was adjusted by red plastic filters.

shows the effect on germination of continuous irradiation by red or far-red light. In this experiment, an incubation temperature of 20 C was employed in order to reduce the germination rate in the dark to an appropriate level. Red light accelerated the germination remarkably, and far-red light was also slightly promotive.

To determine whether a shorter irradiation could be substituted for 12 days of continuous irradiation by red light, the cells were exposed to red light (0.8 $W \cdot m^{-2}$) for various periods of time, 2–12 days, and the germination rates were scored 12 days after the start of exposure. The result is shown in Fig. 6. The germination rate was almost proportional to the irradiation time. This implied that the promotive effect of red light was energy-dependent. Therefore, the relationship between light intensity and the promotive effect was examined (Table 3). Germination was stimulated at all the light intensities tested, and the promotion increased with light intensity. Thus, the red-light effect on germination was dependent on both the duration of irradiation and the intensity of light.

The experiment shown in Table 4 was designed to test whether the cells possessed any specifically light-sensitive stage in the germination process. A 4-day irradiation was given at different times during the 13-day incubation period, but no significant difference was found in germination rates. This meant that no particularly light-sensitive stage existed in the germination process.

Table 4. Effect of red-light irradiation given at different phases of germination[1)]

Irradiation	Germination (%)
Dark, continuous	14±3
Red, 1 – 4 days	50±9
〃 , 2 – 6 days	33±6
〃 , 4 – 8 days	45±9
〃 , 8 –12 days	56±4

1) After preincubation for 3 days at 17 C in the dark, the cells were irradiated with red light (0.8 $W \cdot m^{-2}$) for 4 days at different phases. The germination rates were determined after 12-day incubation at 20 C.

To understand the nature of this red-light promotion, the wavelength dependency of germination was examined with a high energy monochromator. The cells were irradiated for 5 days at 25 C. The intensities of the monochromatic lights employed were not exactly equal, but were within the range of 1.3–1.6 $W \cdot m^{-2}$. The result is shown in Fig. 7. The highest promotion was observed around 625 nm. Blue light

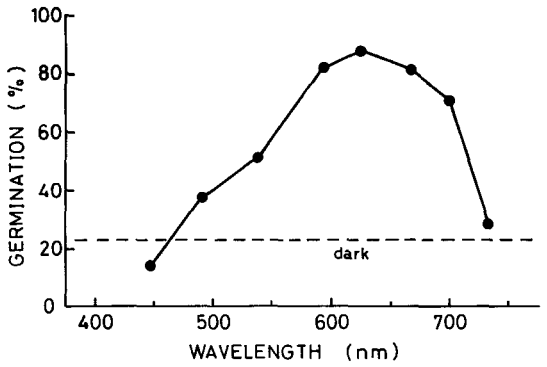

Fig. 7. Wavelength dependency of germination. Cells were irradiated with monochromatic light at various wavelengths for 5 days at 25 C after preincubation for 3 days at 17 C in the dark. The intensity employed was 1.3 $W \cdot m^{-2}$ (447, 538 and 732 nm), 1.4 $W \cdot m^{-2}$ (625 nm), 1.5 $W \cdot m^{-2}$ (472, 598 and 700 nm) or 1.6 $W \cdot m^{-2}$ (669 nm). Broken line indicates the control in the dark. The rates of germination were determined with lots of 160 cells.

Table 5. Effect of DCMU on germination under red- or blue-light irradiation[1)]

Treatment	Germination (%)
For 8 days at 20 C	
Dark, ESP-medium	11±4
Red, ESP-medium	76±6
〃 + 0.025% ethanol	74±2
〃 + 〃 + 10^{-5}M DCMU	13±9
For 6 days at 29 C	
Dark, ESP-medium	39±8
〃 + 0.025% ethanol	38±5
〃 + 〃 + 10^{-5}M DCMU	27±4
Blue, ESP-medium	37±4
〃 + 0.025% ethanol	49±6
〃 + 〃 + 10^{-5}M DCMU	17±3

1) The newly-formed cells were precinbuated for 3 days at 17 C in the dark, then treated in the indicated medium. The light intensity was 0.73 (red), or 0.8 (blue) $W \cdot m^{-2}$. DCMU was originally dissolved in ethanol and diluted with ESP-medium.

(447 nm) appeared to cause a slight inhibition. Assuming that photosynthesis was involved in the photo-promotion of germination, both red and blue light were expected to be promotive. To test the possible participation of photosynthesis, the effect of DCMU on the photo-induced germination was examined. The cells were treated with DCMU for 8 days (under red light, 20 C) or 6 days (under blue light, 29 C), and their germination rates were determined. The results are summarized in Table 5. The presence of ethanol, which had to be added to enhance the otherwise poor water-

solubility of DCMU, made the results a little complicated. However, it was clear that red-light induced germination. Furthermore, DCMU reduced the germination significantly under blue-light irradiation, though 10^{-5}M DCMU appeared a little inhibitive to germination in the dark. This result suggests that photosynthesis also participates in the germination process under blue-light irradiation.

Polarity induction by blue light

During the culture experiments, it was found that the unilaterally irradiated round cell, tended to form rhizoidal protrusions from the shaded part. A cell-axis in the newly-formed, spherical cell was determined by the protrusion of the rhizoid. Thus, the determination of a protrusion site may be regarded as a polarity induction in the round cell.

For convenience, the photo-induction of polarity was estimated as follows: The germinating cells were shadowgraphed at a tenfold magnification, projected through the rectilinear chamber described in the Materials and Methods section. The direction

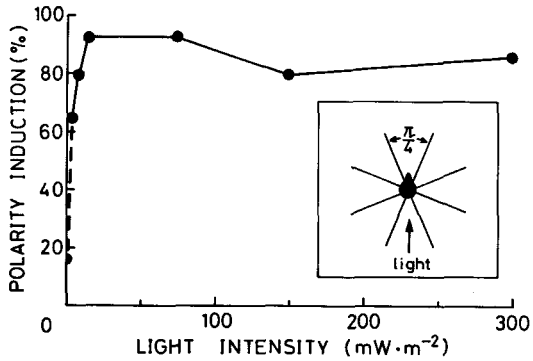

Fig. 8. Relationship between light intensity and the rate of polarity induction. Cells were subjected to continuous, unilateral irradiation with white light for 5 days at 28 C. The arrow indicates the direction of unilateral irradiation. For further explanation of the induction rate, refer to the text. The rates were determined with 40 cells (N=40).

Table 6. Wavelength dependency of the polarity induction[1)]

Wavelength (nm)	Polarity induction (%)[2)]
dark	18
398	40
430	78
467	60
502	32
598	19
664	20
730	18

1) Cells were irradiated unilaterally with monochromatic lights of various wavelengths, for 5 or 7 days at 28 C. The wavelengths tested and their intensities were 398 nm (0.24 W·m^{-2}), 430 nm (0.33 W·m^{-2}), 476 nm (0.29 W·m^{-2}), 502 nm (0.30 W·m^{-2}), 598 nm (0.26 W·m^{-2}), 664 nm (0.25 W·m^{-2}) and 730 nm (0.11 W·m^{-2}).

2) The rate was determined with a lot of 40 cells.

of the outgrowth was measured from the shadowgraphs. The polarity induction was expressed as $n/N \times 100$ (%), where N=total number of germinated cells, n=number of cells whose outgrowths were within the shaded area of $\pi/4$ radians as illustrated in Fig. 8.

The relationship between white light intensity and induced polarity is shown in Fig. 8. In the dark, the rate was 15.6%, which might be interpreted as a random distribution of the protrusion direction. The inducing effect of white light was saturated at the low intensity of 10 $mW \cdot m^{-2}$.

Next, the dependency of this photo-induction upon wavelength was examined with a monochromatic light sources. The cells were irradiated unilaterally for 5 or 7 days at 28 C, and the results shown in Table 6. The intensities of the monochromatic lights were approximately equal, except for that of 730 nm. The results showed a high efficiency in the blue light region, and practically no effect at red or far-red region. It was interesting that red light, which promoted germination remarkably, was ineffective in polarity induction.

Discussion

The strong regenerative activity of *Boergesenia* protoplasm fragments liberated in sea water was probably related to the coenocytic structure of this plant, as a similar activity was observed in *Bryopsis* (Tatewaki and Nagata, 1970) and in some other marine coenocytic algae we had examined, *Valonia ventricosa*, *V. macrophysa*, *Valoniopsis pachynema* and *Boodlea coata* (unpublished). The nature of spheration and regeneration have not yet been clarified in detail, but two characteristics of this process were found: a remarkable temperature dependency and an essentiality of Ca^{2+} in the medium. The essentiality of a rather high concentration of Ca^{2+} in the medium might be related to cell membrane formation in the extracellular protoplasm fragments. Intraprotoplasmic flow is involved during the spheration process, in which some sort of contractile protoplasmic strands seemed to participate. Such a structure was frequently observed in the process of intracellular spheration (Enomoto and Hirose, 1972). As the spheration was not inhibited by dinitrophenol, cyanide or NaN_3, this process did not seem to be directly related to respiration. The notable acceleration of spheration at the high temperature may be explained by a reduction of protoplasmic viscosity. In any case, further experimentation is necessary for an understanding of the spheration process.

The round protoplasts began to regenerate cell walls, which could be detected first with ruthenium red staining, and a little later, with calcofluor staining or by birefringence. At an early stage the cell wall was broken with pectinase treatment. The regenerated cell wall was, therefore, composed mostly of pectin-like polysacchrides at first, and then reinforced with cellulose microfibrils. The presence of cellulosic microfibrils and other characteristics of *Boergesenia* cell walls will be reported elsewhere.

Under the present culture conditions the first step in the growth of the newly-formed cell was the protrusion of a rhizoid, not the expansion of the round cell. The

germination proceeded rapidly at rather high temperatures and was interrupted at temperatures below 17 C. These features of temperature dependence correspond well with the seasonal variation of *Boergesenia* population in the subtropical sea of Amami-ōshima (Enomoto and Hirose, unpublished).

There are few reports in which the effects of plant growth regulators which are known to work in higher plants are clearly demonstrated upon algae (Provasoli and Carlucci, 1974). As summarized in Table 2, the exogenous application of the regulators more or less influenced the germination of this alga. This implied that *Boergesenia* plant possessed receptive sites in the cell for these growth regulators. But, this is another problem and needs further consideration as to whether they are playing any endogenous role in *Boergesenia*.

For the germination of new cells, light exposure was not necessary, but red light promoted the germination significantly. It is well known that the germination of many seed and spores are phytochrome-controlled (Black, 1969; Mohr, 1972). In the case of *Boergesenia*, far-red irradiation produced no or only a slight promotion of germination. Furthermore, this slight promotion seems to be explained by red-light contamination in the "far-red" light employed, as stated in the Materials and Methods section. In this experiment, no clear evidence was obtained to indicate the participation of phytochrome in the germination process.

Another possible mechanism for red-light promotion was the stimulation of photosynthesis, which was reported to be involved in some germination processes (Towill and Ikuma, 1973). The fact that the promotive effect of red light increased with the light intensity or with the irradiation time, and that it was diminished by DCMU, strongly indicated the participation of photosynthesis.

Assuming that germination was promoted through photosynthesis, blue light should exert a promotive effect on the germination similar to the promotive effect of red light. However, blue-light irradiation exhibited no significant promotion, but rather a slight inhibition of germination (Table 5 and Fig. 7). In addition, germination under blue light was significantly reduced by DCMU. These facts suggest that blue light has a dual effect, a promotive effect through photosynthesis and an inhibitive one through another photoreaction.

The location of the rhizoidal protrusion was determined by direction of blue-light irradiation. A similar polarity induction has been reported with *Fucus* eggs (Hurd, 1920; Jaffe, 1958a; Meyer zu Bentrup, 1963), *Botrytis* spores (Jaffe and Etzold, 1962), *Osmunda* spores (Jaffe and Etzold, 1962), *Funaria* spores (Jaffe and Etzold, 1965) and some others (Jaffe, 1958b; Banbury, 1959; Haupt, 1965). The range of wavelengths effective for the induction of polarity in *Boergesenia* was in good agreement with the effective range in the other plants described above.

A blue-light inhibition of *Pteris vittata* spores was reported by Sugai (1971), who suggested that carotenoids might be the photoreceptor pigment. The action spectrum, showing maximum at 450 nm, was similar to that of the polarity induction mentioned above and to that of the phototropism of *Avena* coleoptiles (Thimann and Curry,

1960). Therefore, a rather universal blue-light reaction system seemed likely operating in the polarity induction in *Boergesenia*. Recently, a flavoprotein has been presented as an attractive candidate for the photoreceptor pigment in this reaction system (Briggs, 1976).

The induction of polarity observed in *Boergesenia* cell may be regarded as resulting from the blue-light suppression of rhizoidal growth on the illuminated side of the cell. Assuming that blue light had a growth-suppressing effect on the *Boergesenia* rhizoid, then germination, the initiation of rhizoidal growth, was also suppressed by blue light. In germination, blue light may have exerted two effects, a promotive one through photosynthesis and an inhibitive one through the common blue-light reaction system mentioned above.

However, blue light is now well known to influence phytochrome-mediated physiological responses such as pea stem elongation (Bertsch, 1963) and rice coleoptile growth (Pjon and Furuya, 1967), where mutual reversibility between blue and far-red light was demonstrated, and growth in *Lunularia cruciata* (Schwabe and Valio, 1970), in which the reversibility between blue and red light was demonstrated. Since the effect of far-red or red light upon the blue-light inhibition in *Boergesenia* has not yet been tested, the possibility can not be excluded that phytochrome is involved in the germination or polarity induction process. *Boergesenia* rhizoid was found to exhibit a sensitive negative phototropic bending upon blue light. The participation of the phytochrome system was distinctly shown by the phototropism of this alga, which will be reported elsewhere.

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