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RHIZOID DIFFERENTIATION IN SPIROGYRA

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I. Basic features of rhizoid formation

Rhizoid differentiation in Spirogyra I.

Basic features of rhizoid formaiton

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Summary

Several types of rhizoids in the process of differentiation in Spirogyra sp., probably Spirogyra fluviatilis, were described and their interrelation was elucidated. There are two main differentiation sequences, that is, $P_p \rightarrow P \rightarrow Rh_{ros}$ or $P_p \rightarrow P \rightarrow Rh_{rod} \rightarrow Rh_{ros}$ (for explanation of abbreviations see p. 6 and 7), although under some conditions the sequences ceased halfway. The initiation time of the rhizoid formation had no relation to the stage of the cell cycle. The difference in the growth pattern between the rhizoid and the ordinary filament cell was demonstrated with Calcofluor-staining and centrifugation.

The optimal temperature and pH of the culture medium for rhizoid differentiation were 20°C and pH 7, respectively. It was demonstrated that contact stimulus was not necessary for the induction.

Of several environmental factors examined light was most important for rhizoid formation, since rhizoid was induced only when light was given after cutting the filament.

Introduction

It is well known that filaments of some species of Spirogyra growing in a stream attach to the bed with their rhizoids. They attach also to the wall of the culture vessel in the laboratory forming the rhizoids. They take several shapes, namely, small primordial protuberance, larger protuberance, rod, and rosette. The differentiation of the rhizoids is characterized by its rapid development after appearance of a small protrusion of the cell wall. It is a problem which attracts our attention how a part of the ordinary vegetative cell comes to differentiate into a rhizoid without cell division.

In spite of many publications on Spirogyra, papers dealing with the development of rhizoid are not many. Most of these papers are taxonomical or morphological ones and only a few are concerned with the effective stimuli inducing formation of rhizoids (1). The only paper dealing with the cytology and physiology of the rhizoid cell in detail was presented by Konrad von Weihe (1), who maintained that the rhizoid was initiated by a local change of surface tension of the cell.

In the present paper the process of the rhizoid genesis was described and the growth pattern of the rhizoid was compared with that of the ordinary filament cell. Next were investigated stimuli inducing rhizoid formation. Among several environmental factors affecting the rhizoid formation, light seemed to be most important, because the rhizoid could not be formed in the dark while it was produced in light. The effect of light on rhizoid formation will be described separately in the second paper (2) of this series.

Material and Methods

Spirogyra sp.[1] was collected from the stream near Osaka University (Toyonaka, Osaka) about five years ago. The alga has been cultured in a medium[2] slightly modified in our laboratory from Reichart's inorganic medium (3) (pH 7.2) in a growth cabinet at 20°C, under 12 hr - 12 hr light-dark cycle at 2,500 lux. Besides the modified Reichart's medium Darden's medium (pH 7.0) (4) was also used occasionally. There was, however, no noticeable difference in experimental results between the two media. The experiments were done using axenic culture which was obtained by the method of Ooiwa (in preparation). For illumination white fluorescent light was used.

For preparing specimens ten to twenty filaments sandwiched between two agar blocks in parallel with one another were cut into pieces about 0.9 mm long with a cutter

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[1] Tentatively identified as *Spirogyra fluviatilis* by Dr. T. Yamagishi. The cell with the diameter of about 33 μm contained three or four chloroplast bands.

[2] One liter of the medium contained 200 mg of KNO_3, 15.6 mg of KH_2PO_4, 10 mg of H_3BO_3, 6.6 mg of $FeSO_4 \cdot 7H_2O$, 8.85 mg of $2Na \cdot EDTA$, 100 mg of $NaHCO_3$, 4 mg of $CaSO_4$, 10 mg of $MgSO_4 \cdot 7H_2O$, 5 mg of $MnCl_2 \cdot 4H_2O$, 0.5 mg of $ZnSO_4 \cdot 7H_2O$, 0.1 mg of MoO_3, 2 μg of $CoCl_2 \cdot 6H_2O$, 500 mg of Tris, 3.5 ml of 1 N HCl and distilled water.

made of a pair of razor blades (Fig. 1). A short segment of filament thus cut contained 3 - 8 cells. A set of the filaments obtained in this manner were introduced into van Tieghem's cell, 1.6 cm in diameter, containing the culture medium, so that each van Tieghem's cell contained a complete set of filaments from the same origin.

The experimental procedure for obtaining the rate of rhizoid formation was the same as that used in the subsequent work (2) dealing with the effect of light on rhizoid formation. It consisted of preincubation in the dark lasting 24 hr, illumination with white light for a short period, and postincubation in the dark lasting 16 to 24 hr. Thereafter cells with rhizoids in all the developmental stage were counted for each van Tieghem's cell under an inverted microscope. The rate of rhizoid formation is expressed as percentage of the number of rhizoid cells to the total number of terminals of the filaments in a van Tieghem's cell. It should be mentioned here that both terminal cells of a segment of filament can produce rhizoids equally well and therefore the filament is likely to be isopotent.

In order to see the growth pattern of the cell wall, filaments of Spirogyra were incubated in 0.05% solution of Calcofluor White ST (American Cyanamid Company, Bound Brook, N.J.) at 20°C. Then the material was washed first with H_2O and subsequently with Calcofluor-free culture medium 3 or 4 times. Thereafter, it was brought into the culture medium at 20°C for several days and observed under a fluorescence microscope (for details, see results).

Centrifugation was performed in two different ways. In one case, segments of Spirogyra filaments were centrifuged continuously for as long as 5 to 17 days. Culture of the segments under continuous centrifugation was carried out after Kamiya (unpublished). The segments were embedded in 1% agar gel in such a position that the segments were centrifuged longitudinally. The magnitude of acceleration was 400 × g and the material was illuminated 8 hr a day at 1,000 lux with a fluorescent tube at room temperature. In the other case the preincubated segments sandwiched between a pair of wet pieces of the pith of elderberry were centrifuged longitudinally at 7,000 × g, for 15 min with a refrigerative centrifuge (Kubota, KR-6P) at 0°C[1] using a swing-type bucketrotor. Thereafter the filaments were illuminated at 1,000 lux for 5 min and postincubated in the dark for 16 - 24 hr at 20°C before observation.

The temperature during the experiments was 20°C, unless it is described otherwise.

Results

Process of rhizoid formation

The terminal cells of the filament of Spirogyra used for the present work readily form rhizoids under the normal culture condition. Several types of rhizoids are observed. They are classified into four types, namely, primordial

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[1] After centrifugation all the segments were first brought into a small Petri dish with the cold culture medium in the centrifuge tube and thereafter they were divided into several van Tieghem's cells with the culture medium at 20°C. If the temperature of the medium in the Petri dish was not cold, many segments attached to the glass. This made transfer of the segments into van Tieghem's cells difficult.

protuberance (P_p), papillary protuberance (P), rod-shaped rhizoid (Rh_{rod}) and rosette-shaped rhizoid (Rh_{ros}). Detail description for these types and the relations between them will be described below.

The process of rhizoid formation was observed under the following condition. The filaments cultured in a growth cabinet under a 12 hr - 12 hr light-dark regime were cut and preincubated in the dark for 24 hr. Thereafter, they were cultured in the growth cabinet again. Duration of the first light period after the preincubation did not affect the process of rhizoid formation. The result is shown in Figure 2. Arabic figures in parentheses in the figure show approximate periods of time necessary for the formation of rhizoids after the onset of illumination. Photomicrographs of these rhizoids were also given in Figure 3. As soon as the filament was cut, the distal septum of the terminal cell was exposed to the external medium and became convex (Figs. 2 and 3A). Five hours after the filament was cut, the walls of the cut cell, including its side wall and a half layer of the cross wall, were detached from the terminal cell (Figs. 2 and 3B).

On illumination of the filaments after 24 hr incubation in darkness (Figs. 2 and 3C), many terminal cells adhered soon to the substrate at their tip. This can be confirmed by stirring the medium with shaking. A small primordial protuberance (P_p, Figs. 2 and 3D) was observed at the tip about three hours after the onset of illumination. During the subsequent growth of the protuberance, the chloroplasts invaded into it from the basal cylindrical part of the cell.

This stage of protuberance is designated as papillary protuberance (P, Figs. 2 and 3E). Up to this stage it was observed that some of the cells attaching to the glass detached from it and inversely some of the free terminal cells attached to the glass. The adhered papillary protuberance, P, elongated to a single rod-shaped rhizoid (Rh_{rod}, Figs. 2 and 3F) or to branched rod-shaped rhizoids (Rh_{rod}, Fig. 3G), or without elongation it became a rosette-shaped rhizoid (Rh_{ros}, Figs. 2 and 3H). Some of the adhered Rh_{rod} transformed to Rh_{ros} (Figs. 2 and 3I) forming a disk-like plate at the tip or on the side of the rod-shaped rhizoid. The unattached protuberance (P) elongated and differentiated to free Rh_{rod}. A vigorous protoplasmic streaming was observed at the distal region of the rhizoid in which the growth of the wall was so fast that the invasion of the chloroplasts could not follow. The adhesiveness to the substrate of the protuberance and of the rhizoid of more advanced stage (Rh_{rod}) was delicate, that is, sometimes attached cells detached from the glass or free rhizoids attached to it. On the contrary, Rh_{ros} was formed only when the cell adhered to the substrate. It never detached from the substrate.

Rod-like rhizoid cells differentiated further to branched Rh_{rod} (Fig. 3G). Similarly, Rh_{ros} which was smooth in contour, enlarged its area leaving many incisions so that it looked like a flower of chrysanthemum (cf. Fig. 3I). The rhizoid, which did not differentiate into Rh_{ros}, detached from the substrate so that a few months later only rosette-type rhizoids remained attached to the culture vessel. Thus the Rh_{ros} was supposed to be the last developmental form of the terminal cell which attached to the substrate. At this final stage the chloroplasts found mostly in the non-rhizoidal part were divided into smaller fragments.

The cell from which a rhizoid differentiated, or simply the "rhizoid cell", exhibited several characteristic features. First, the rhizoid cell lost the ability to divide. For example, out of 131 rhizoid cells 37 cells (28 %) divided at P-cell stage, 7 cells (5%) at Rh_{rod}-cell stage and none (0%) at Rh_{ros}-cell stage. In parallel with the loss of the ability of cell division the rate of elongation of the non-rhizoidal part of the cell decreased and Rh_{ros}-cell never elongated. When the rhizoid was formed from the cell stained with Calcofluor beforehand, the intensity of fluorescence of the non-rhizoidal part was kept without fading whereas the fluorescence of the ordinary filament cell was weakened as the cell elongated and divided. Second, the rhizoid cell older than a few days raised its osmotic pressure. In one case it was observed that in 0.5 M mannitol solution the rhizoid cell did not plasmolyze, though the filament cell did in that solution. Third, with the development of the rhizoid, chloroplasts which were originally spiral loosened and oriented themselves in parallel to the cell axis except at the vicinity of the cell nucleus. This tendency was first discernible at the P= stage. Therefore the rhizoid cell looked pale green in comparison with the undifferentiated non-rhizoidal cell (Fig. 3J). All these marked features are characteristics of the cell from which the rhizoid differentiated.

Sometimes rhizoid was produced from the side wall of the terminal cell (Fig. 3K). It also happened that one and the same cell formed two rhizoids, one at the distal end and the other on the side wall. The side-rhizoid was observed at a considerably high rate (more than 90%) in a gelatin solution (2%). In the normal culture medium, for example, it was formed in the terminal cell to which the wall of the adjacent cut cell was still attached.

It has hardly been observed that the cells other than the terminal cells produced rhizoids without the terminal cells having formed rhizoids. When the rhizoid was formed in the second cell from one of the terminal cells of a filament, it was observed in one case that the terminal cell had already differentiated into a rhizoid cell, and in another case that the terminal cell was injured. Rarely the terminal cell first formed a rhizoid from the proximal side wall and then divided into two daughter cells so that the proximal one was provided with the rhizoid and the new terminal cell remained without rhizoid.

Rhizoid formation versus cell cycle

Under the axenic culture conditions one cell cycle lasted 2 or 3 days. During this period of time cell length reached more than 200 µm. The cell length may be a parameter for the stage of the cell cycle. It was intended to know whether or not there is a relationship between the stage of the cell cycle and the rhizoid formation. In Table 1 rate of rhizoid formation was shown in relation to the length of the cells which just began to form P_p. The result shows that cells of various lengths could all form a rhizoid, where shorter cells appeared to form it more readily than longer ones.

Differences in growth pattern between rhizoid and ordinary undifferentiated cell

The rhizoid grew by apical growth while the ordinary cell grew uniformly over all the cell wall. This was elucidated by the staining experiments, using Calcofluor, a fluorescent dye having a high specificity to cellulose (5). In the experiment of Figure 4, the undifferentiated cell, preincubated in the dark for 24 hr, was stained in 0.05% solution for 30 min. After staining it was incubated in the culture medium under normal culture condition for 2 days. The photomicrograph in the figure shows that there is a distinct difference in the intensity of fluorescence between the non-growing (intense) and the growing (weak) parts. As control, rhizoidal part which had been formed before the staining was intensively stained. Similar results were obtained when P and Rh_{ros} were stained and cultured. The above observation demonstrated that throughout the rhizoid differentiation only the distal region of the rhizoid grew.

Growth pattern of the ordinary cell was also studied by labelling the filament with 0.01% Calcofluor for 17 hr at 20°C. After subsequent culture of the labelled filament for a week in the culture medium, the filament was centrifuged for a short period of time to have the chloroplasts evacuate from the centripetal end so that the cell wall of that part could be well observed. The fluorescence emitted from the wall was weakened progressively and evenly over the whole length of the cell as the cell grew (Fig. 5). From this result it was suggested that in the ordinary filament cell, elongation was not localized in a particular region but occurred uniformly over its entire length.

Second, the rhizoid grew in a condition in which the ordinary cell and the non-rhizoidal part of the rhizoid cell did not. For example, even in continuous darkness subsequent to a brief illumination, P was formed and differentiated to Rh_{rod} or Rh_{ros}. When the filament was under a continuous centrifugal force the rhizoid was formed and grew, while the non-rhizoidal part of the rhizoid forming cell and the ordinary cell did not. It is to be noted that the rate of formation at the centripetal end was considerably higher than that at the centrifugal end of the cell. On the average of fourteen experiments rhizoid was formed at the rate of 42% at the centripetal end and of only 3.8% at the centrifugal end.

Third, the terminal cell, whose chloroplasts and other cell constituents were dislocated through brief centrifugation, formed the rhizoid at the centripetal end (Fig. 6) and at the centrifugal end equally well. Fujii (personal communication) investigated growth of a centrifuged cell of another undefined species of Spirogyra which did not form rhizoid. She found that the cell hardly elongated at the centripetal end of the cell whilst it grew well at the centrifugal end in which the chloroplasts and other cell constituents were accumulated. Thus, in the growth mechanism there may exist a distinct difference between the rhizoid and the non-rhizoidal cell.

Factors affecting rhizoid formation

1. Temperature

The temperature was kept at 0, 15, 20 or 30°C in the experiment during both preincubation and postincubation (Table 2). Deviation of temperature from 20°C either upwards or downwards affected significantly the rhizoid formation and an extremely low temperature inhibited it perfectly. The optimum temperature for rhizoid formation was 20°C.

2. pH

The cut segments were kept during preincubation, illumination and postincubation in the Darden's medium adjusted at various pH. The result in Table 3 shows that the maximal rate of rhizoid formation was found at pH 7.

3. Contact stimulation

That contact is essential in *Spirogyra* to induce formation of the rhizoid has been pointed out repeatedly (1). To confirm this the following experiment was carried out. The filament was held by embedding one end into agar and was hung in the medium before the filament was cut short so that the other end never had a chance to make contact with the substrate (Fig. 7). Then, the hung filament was preincubated, illuminated at 2,500 lux for a short period of time and postincubated. Under such a condition they formed P-type rhizoids in their free ends. The rate of rhizoid formation of the free ends amounted to 36% on the average of 7 separate experiments.

The other series of experiments were done by suspending the preincubated segments in density-graded Ficoll solution in a van Tieghem's cell. They suspended at the boundary between the 7% and 14% layers without having come in contact with the vessel wall. The suspended filaments in the Ficoll solution were illuminated and postincubated. It was observed that they formed protuberances (P_p, P) and rod-shaped rhizoids (Rh_{rod}) (the formation rate, 50%, on the average of 3 separate experiments) but did not form rosette-type rhizoid. Thus it may be concluded that without any contact stimulation rhizoids other than Rh_{ros} can be formed and that contact stimulation is needed for Rh_{ros} formation.

4. Light

If the cut filament was not illuminated at all, the terminal cell never formed the rhizoid as shown in Table 4. The segments were illuminated with a light of 1,000 lux continuously for 24 hr. It is clear that illumination of the filaments is indispensable after cutting for rhizoid formation but not necessary before cutting.

Discussion

In the filament the rhizoid formation was restricted always only to the terminal cells. Based on the present results with a light microscope, it is possible that the dome-like projection of the terminal septum may be necessary for the cell to perceive that the cell is in the terminal position. Further it is probable that some submicroscopic events such as rearrangement of cell organelles take place in the very early stage where no microscopic changes are yet detectable such as is the case in the formation of rhizoids in the *Fucus* zygote (6) and *Polysiphonia* (7).

The importance of the contact stimulus for the rhizoid formation has already been described. Czurda (cf. (1)) gave an expression that the formation of the differentiated "Haftorgane" in Zygnematales is a sort of thigmo-morphogenesis. Weihe (1) maintained that the stimulus inducing the rhizoid formation (in *Spirogyra fluviatilis*) is in effect a certain difference in the surface tension between the cell and the outer medium. The theory was deduced from the following two experimental facts. First, the filament, which was hung in a medium so that its free end did not come in contact with a substrate, did not form the rhizoid but the one hung in moist air produced the rhizoid. Second, the filament suspended in the solutions of agar, gum arabic or urea formed the rhizoid, only when their concentrations were made higher above definite levels. He assumed that various carbohydrates, alcohol and asparagine which acts to stimulate rhizoid formation could alter the surface tension, and he also interpreted that the contact stimulus will give rise to changes in the surface tension of the cell. The result obtained in the present experiment that the segments suspended in the Ficoll solution formed the rhizoids, may be explained by his theory. However, the fact that the filament hung in the culture medium also produced the rhizoid in its free terminal cell is contrary to the result obtained by Weihe.

Further, Weihe reported that cells smaller than 120 μm could not form the rhizoid. He concluded that for the formation of the rhizoid the ratio of the surface area of the side wall to the area of the septum should exceed a certain value, that is, 14. In the present experiment, however, short cells smaller than 120 μm could also produce rhizoids as well as longer cells (cf. Table 1). Moreover, the rhizoid was formed frequently in the cells attaching to the substrate with its tip but not with its side wall. Thus the ratio of the surface areas between the side wall and the septum, that is, the extent of the contact of the side wall to the substrate, seems to have no relation with the rhizoid formation.

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References

(1) Weihe, K. von: Über die Rhizoidbildung und die Rhizoidzelle bei *Spirogyra*. *Zeitschr*. *Bot*. 40: 231-249 (1952).

(2) Nagata, Y.: Rhizoid differentiation in *Spirogyra*. II. Photoreversibility of rhizoid induction by red and far-red light.

(3) Reichart, G.: Die Synchronkultur von *Spirogyra*. *Ber*. *Deut*. *Bot*. *Ges*. 80: 177-186 (1967).

(4) Darden, W. H., Jr.: Sexual differentiation in *Volvox aureus*. *J*. *Protozool*. 13: 239-255 (1966).

(5) Harrington, B. J. and K. B. Raper: Use of a fluorescent brightener to demonstrate cellulose in the cellular slime molds. *Appl*. *Microbiol*. 16: 106-113 (1968).

(6) Quatrano, R. S.: An ultrastructural study of the determined site of rhizoid formation in *Fucus* zygotes. *Exp*. *Cell* *Res*. 70: 1-12 (1972).

(7) Guillard, R. R. L. and C. J. Lorenzen: A light and electron microscopic study of rhizoid development in *Polysiphonia* *lanosa* (L.) Tandy. *J*. *Phycol*. 8: 15-24 (1972).

Table 1. Rhizoid formation in relation to cell length.

Cell Length × 100 μm	1.00 – 1.19	1.20 – 1.39	1.40 – 1.59	1.60 – 1.79	1.80 – 1.99	2.00 – 2.19	2.20 – 2.39	2.40 – 2.59	2.60 – 2.79	2.80 – 2.99	3.00 ∧
Number of Observed Terminal Cells	4	20	45	53	50	33	26	33	30	16	12
Rate of Rhizoid Formation (%)	100	80	74	59	64	55	62	52	67	56	50

The preincubated segments of the filaments were illuminated at 2,500 lux. The length of the cell which had just begun to form P_p was measured with an ocular micrometer.

Table 2. Influence of temperature on rhizoid formation

Temperature	Rate of Rhizoid Formation (%)
0°C	0
15°C	17
20°C	69
30°C	6.4

Excised filaments were preincubated and postincubated each at the same temperature. The material was illuminated with 4,500 lux at 20°C for 20 min between pre- and post-incubation.

Table 3. Influence of pH of the culture medium on rhizoid formation.

pH	Rate of Rhizoid Formation (%)
5	31
6	56
7	72
8	53

Excised filaments were placed in media with different pH during preincubation, illumination (900 lux, for 20 min) and postincubation.

Table 4. Rhizoid formation under various light conditions before and after cutting the filaments.

Light Condition	Rate of Rhizoid Formation (%)
L/L[a]	49
L/D[b]	3.3
D/L[c]	58
D/D[d]	0.6

[a] Before as well as after cutting filaments were kept in light at 1,000 lux for 24 hr.

[b] Before cutting filaments were kept in light at 1,000 lux for 24 hr, and after cutting in the dark for 24 hr.

[c] Before cutting filaments were kept in the dark for 24 hr, and after cutting in light at 1,000 lux for 24 hr.

[d] Before as well as after cutting filaments were kept in the dark for 24 hr. The filaments were cut under safe light.

Each value represents the mean of 4 separate experiments.

Legends for Figures

Fig. 1. A double-blade cutter and the Spirogyra filaments placed in parallel between two agar blocks. B, razor blade; F, Spirogyra filaments; A, agar block.

Fig. 2. A diagram showing the sequences of rhizoid formation of various shapes. P_p, primordial protuberance; P, papillary protuberance; Rh_{rod}, rod-shaped rhizoid; Rh_{ros}, rosette-shaped rhizoid. The numbers in parentheses show an approximate periods of time necessary for the formation of rhizoids after the onset of illumination.

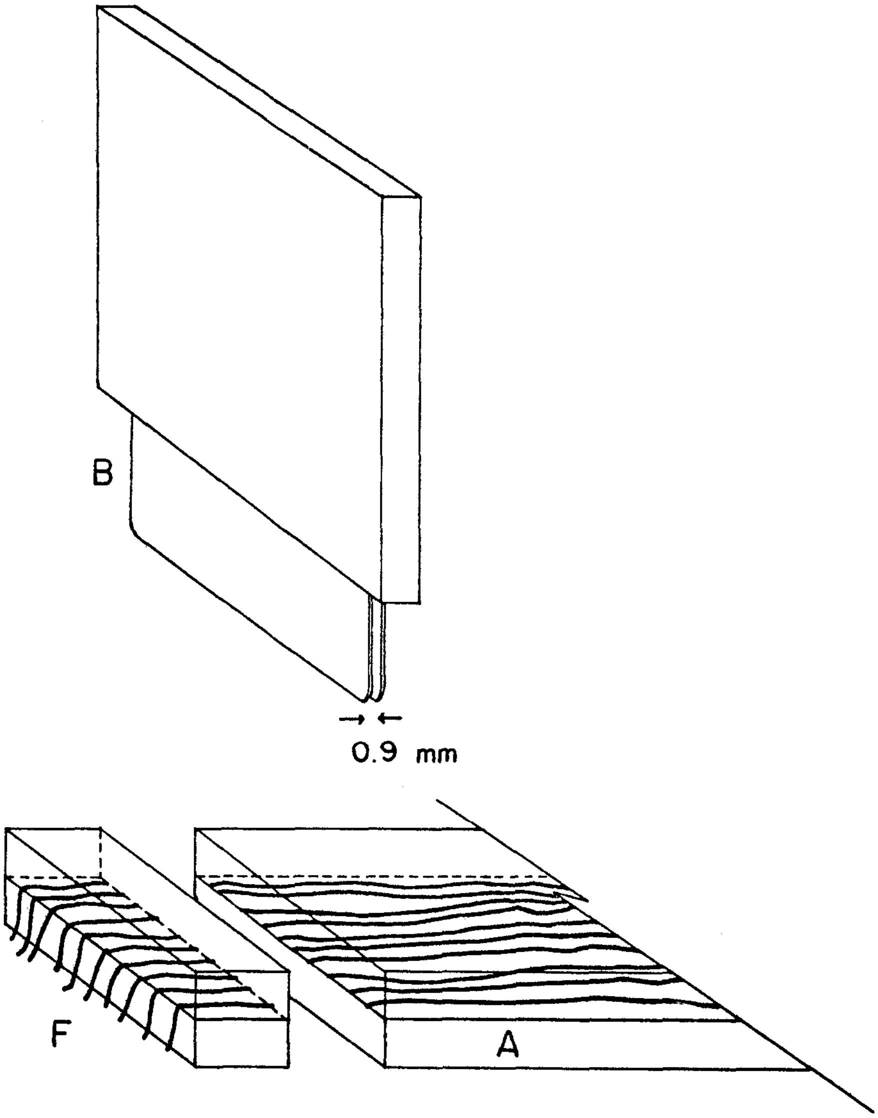
Fig. 3. Photomicrographs showing rhizoids of various shapes. A, a short segment immediately after cutting of the filament; B, detachment of the residual walls of the cut cell from the short segment; C, the short segment at the onset of illumination; D, primordial protuberance (P_p) formed at the distal end of the short segment; E, papillary protuberance (P); F, rod-shaped rhizoid (Rh_{rod}); G, branched Rh_{rod}; H, rosette-shaped rhizoid (Rh_{ros}); I, Rh_{ros} formed from Rh_{rod}; J, a rhizoid (Rh_{rod}) cell containing chloroplasts loosened and oriented in parallel to the cell axis except at the vicinity of the cell nucleus; K, Rh_{ros} produced in the side wall.

Fig. 4. Rh_{rod} formed from the Calcofluor-stained, undifferentiated cell. Top, the specimen photographed under bright field; Bottom, the same picture in fluorescent image.

Fig. 5. Calcofluor-stained normal cell centrifuged a week after the staining. The chloroplasts were accumulated at the centrifugal end of the cell. Top, the specimen photographed under bright field; Bottom, the same picture in fluorescent image.

Fig. 6. Rh_{rod} formed in the centripetal end after centrifugation for 15 min at 7,000 × *g*.

Fig. 7. Diagram of the setup for hanging Spirogyra filaments. Each filament was supported in 1% agar gel in a glass tubing. The glass tubing was inserted into a spongy polystyrol resin which was kept submerged in the culture medium in a glass beaker. T, glass tubing; A, agar gel; F, *Spirogyra* filament; R, spongy polystyrol resin; M, culture medium; B, glass beaker.



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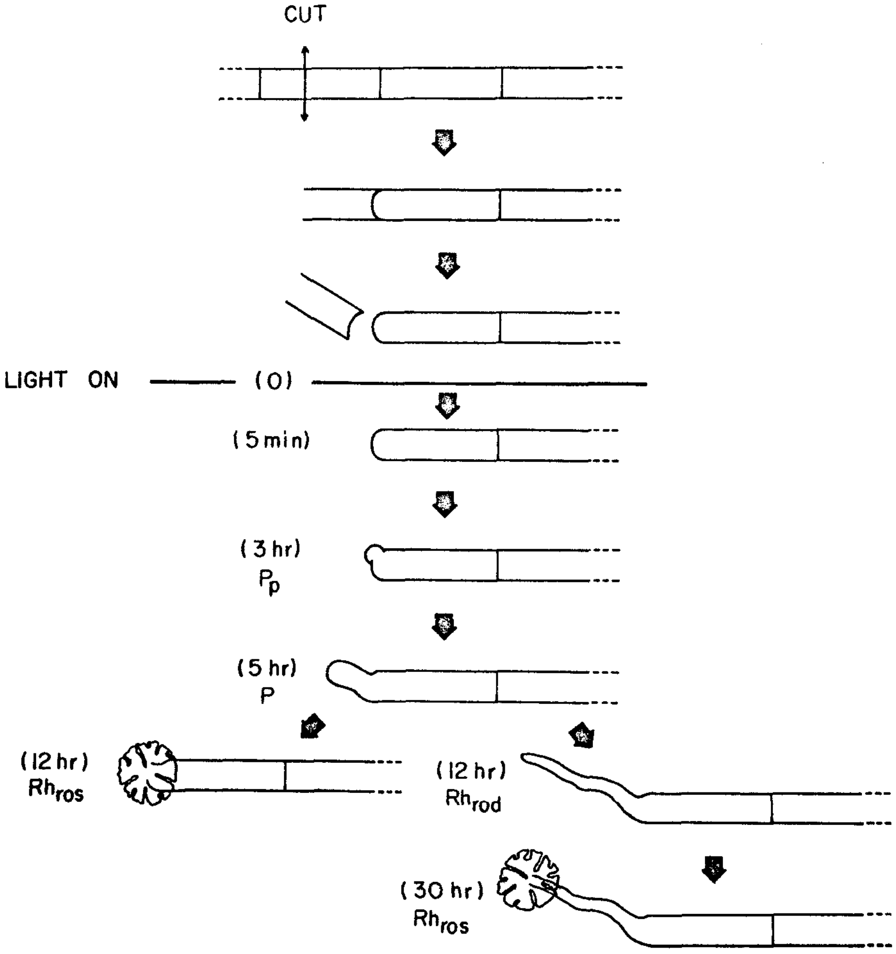

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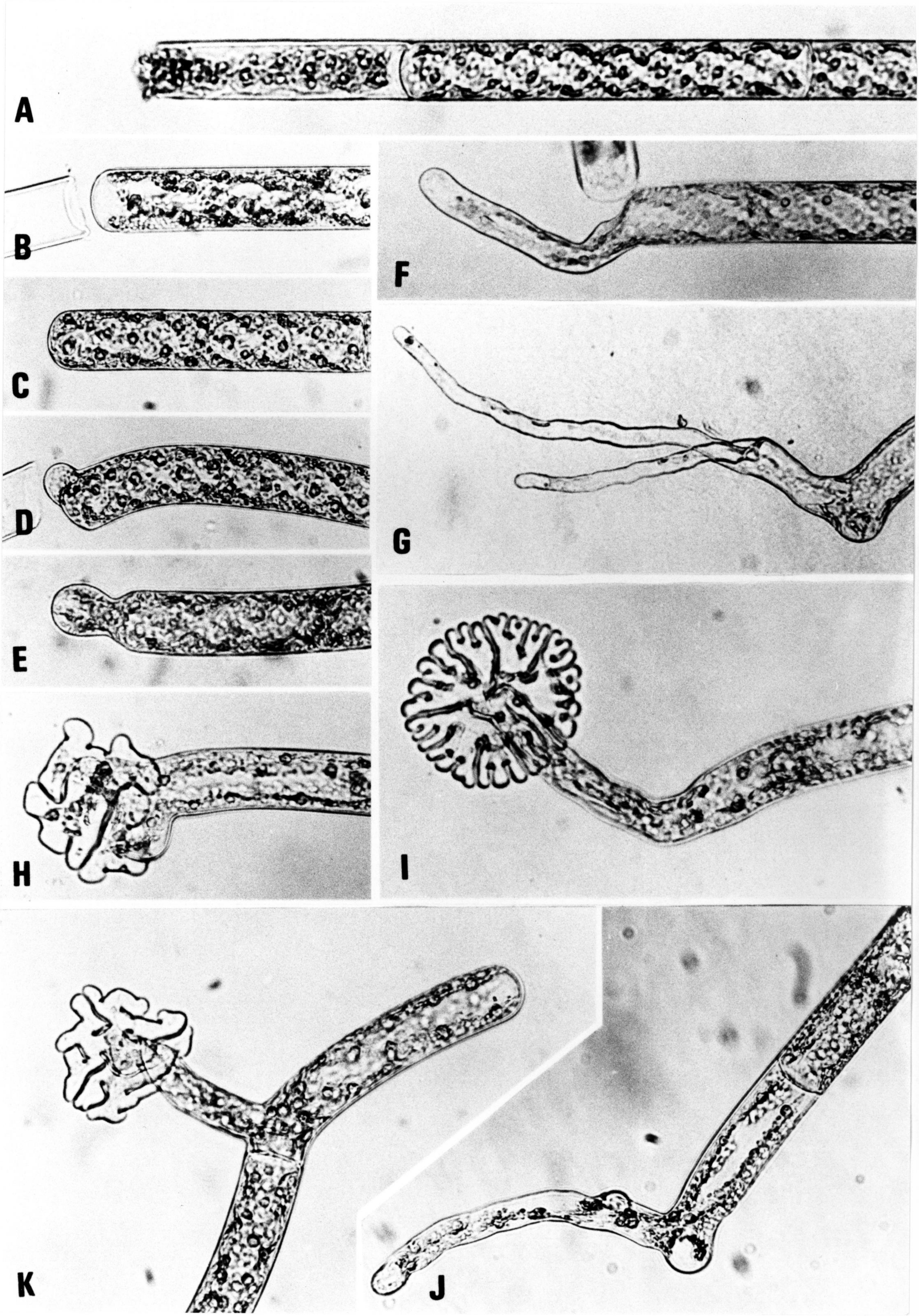
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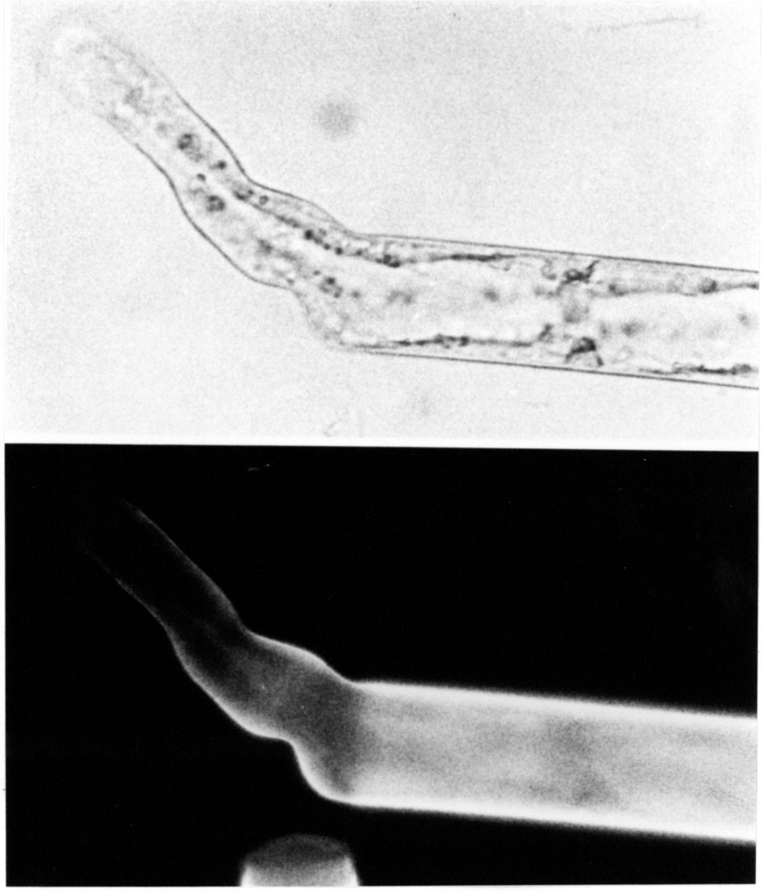

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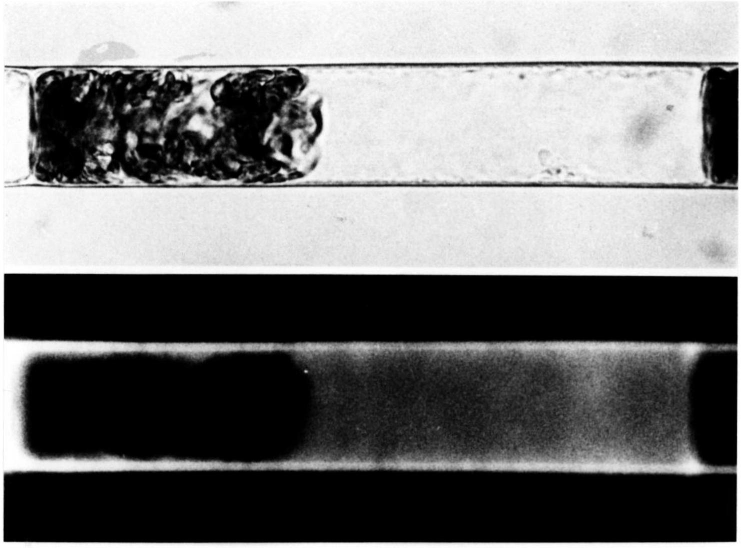

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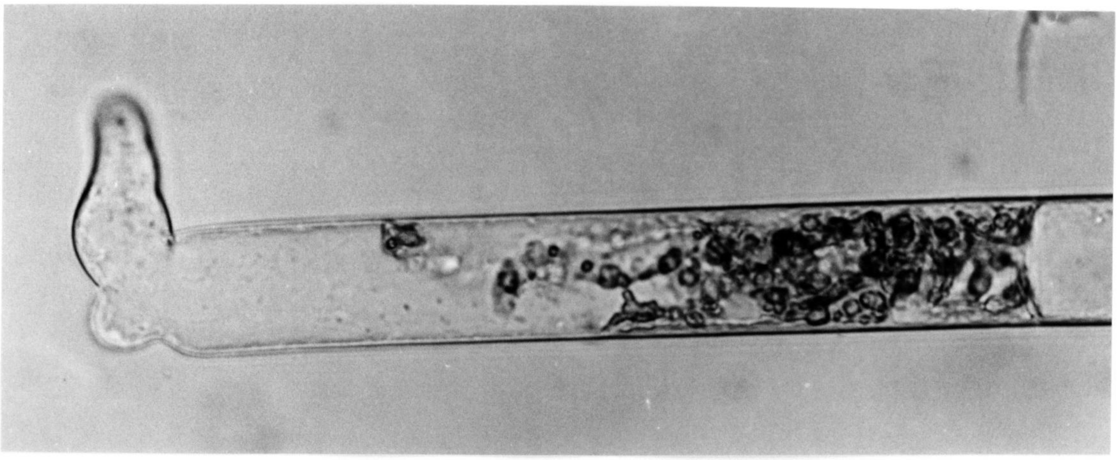

Fig. 6. Rh$_{rod}$ formed in the centripetal end after centrifugation for 15 min at 7,000 × g.

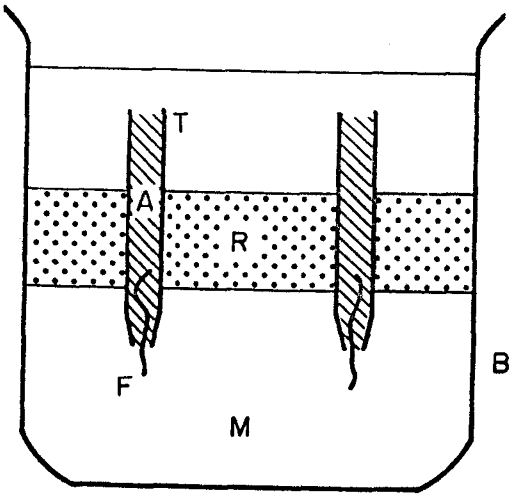

Fig. 7. Diagram of the setup for hanging *Spirogyra* filaments. Each filament was supported in 1% agar gel in a glass tubing. The glass tubing was inserted into a spongy polystyrol resin which was kept submerged in the culture medium in a glass beaker. T, glass tubing; A, agar gel; F, *Spirogyra* filament; R, spongy polystyrol resin; M, culture medium; B, glass beaker.

II. Photoreversibility of rhizoid induction by red and far-red light

Rhizoid differentiation in Spirogyra II.
Photoreversibility of rhizoid induction by red and far-red light

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Summary

The optimal experimental light conditions for rhizoid formation were decided. Red light was effective while green, blue and violet lights had a little effect on the rhizoid formation. The dose-effect curve of red light was investigated and the minimum energy to saturate the effect was estimated to be 8.1 Kergs cm^{-2}. The effect of red light was inhibited by subsequent irradiation of far-red light. The dose-effect curve of far-red light was also obtained. The repeatedly reversible photoresponses with red and far-red light strongly suggests that the photoreceptor of the rhizoid formation system in Spirogyra is phytochrome. The existence of phytochrome in the Spirogyra cell was demonstrated also spectrophotometrically. The half time for the escape reaction from the inhibitory effect of far-red light was obtained to be $2\frac{2}{3}$ hr. There may be no pigment mediating the photoreaction other than phytochrome.

Introduction

Some species of *Spirogyra* growing in a stream attach to the bed with their rhizoids. They attach also to the wall of the culture vessel in the laboratory forming the rhizoids. When a filament of a species of *Spirogyra* was cut, the terminal cells of the excised segments differentiated to rhizoid cells without cell division. The growth of rhizoid was apical while that of the ordinary, filament cell was not locally restricted. Rhizoids in several shapes, namely, papillary protuberances, rod-shaped rhizoids and rosette-shaped rhzoids were formed. All these rhizoids developed from primordial protuberances. The primordial protuberance was produced only when the cut segment was illuminated. Therefore it is known that light is necessary for the formation of rhizoid in *Spirogyra* (1). In many spores of other plants it is known that rhizoid growth is sensitive also to light stimulation (2). In the present study, the author's efforts were devoted to identify the photoreceptor pigment.

Material and Methods

Material and main experimental procedures were the same as the previous study (1). *Spirogyra* sp.[1] has been cultured aseptically at 20°C, under 12 hr - 12 hr light-dark cycle at 2,500 lux. Van Tieghem's cell contained ten to twenty short

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[1] Tentatively identified as *Spirogyra fluviatilis* by Dr. T. Yamagishi. The cell with the diameter of about 33 µm contained three or four chloroplast bands.

segments of the Spirogyra filaments was preincubated in the dark for 24 hr, illuminated with white light or colored light for a short period, and postincubated in the dark for 16 to 24 hr[1]. Thereafter cells which formed rhizoids were counted for each van Tieghem's cell under an inverted microscope irrespectively of rhizoids' developmental stages. The rate of rhizoid formation is expressed as percentage of the number of rhizoid cells to the total number of terminals of the filaments in a van Tieghem's cell.

The safe light used was dim green light. The light source, a 10 W green fluorescent tube (Mitsubishi, FL 10 G), was covered partially with a black paper, in order to prevent the direct illumination on the material. The very weak green light (1 lux) reflecting from the wall of the dark room was enough for experimental handlings. Observation of the material under the microscope was carried out by white fluorescent tubes (Toshiba, FL 32S-W/NL). Colored light irradiations were performed in the following way. As a light source a 500 W incandescent lamp (EYE reflector flood lamp, PRF 500) was used. The van Tieghem's cell was placed in a dark box equipped with a window in which several pieces of filters were fitted. The material in the van Tieghem's cell was then irradiated by the light passing through a 5 cm thick water layer and the filters. For various combinations of the filters the transmission bands were obtained with a recording spectrophotometer (Hitachi, EPS-2) (Fig. 1). The filters used were interference filters for a spectrophotometer

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[1] Refer to Results.

(Hitachi), an interference filter (Toshiba) and glass color filters (Toshiba). Irradiation energies of the colored lights which reached the material were measured with a thermopile (Kipp and Zonen).

A difference absorbance between 650 nm and 710 nm on the irradiation of red or far-red light was obtained with a double-beam recording spectrophotometer (Hitachi, 356 DM).

Results

Preliminary experiments

Although illumination is indispensable for the rhizoid formation, it does not mean that light must be given to the filament continuously. In order to examine how much dosage of light was required for the maximum rate of rhizoid formation, the material incubated in darkness for 24 hr were illuminated with white fluorescent tubes for various periods of time at 1,000 lux (Table 1). The saturation dosage for the maximum rhizoid formation was 30 min at 1,000 lux, that is, 30,000 lux•min. Therefore in the following experiments the material was illuminated with the fluorescent lamp to such a dosage as to exceed 30,000 lux•min with the exception of several experiments.

A question arises how the length of the dark period before illumination (1,000 lux, for 30 min) influences the rhizoid formation. As shown in Figure 2, duration of the dark period needed for the optimum rhizoid formation was different according to the states of the material used for the experiment, namely, 24 hr (curve I), 36 hr (curve II), 48 hr (curve III) and more than 72 hr (curve IV). Although the relation between the length of the dark period and the rate of rhizoid formation varies from material to material, most materials followed curve I in which the optimum rhizoid formation was obtained after 24 hr dark incubation. For this reason the segments incubated in the dark for 24 hr after the cutting were used in the subsequent light irradiation experiments and this dark incubation will be referrred to simply as the "preincubation" in this paper.

The filaments after illumination were incubated in the dark for various periods of time, and the rate of rhizoid formation was determined by counting the number of rhizoid cells detectable under microscope. In one experiment, the counting of the rhizoids was performed in one and the same sample at different times during the dark incubation under the safelight, that is, dim green light (Table 2). In the other set of experiments the counting was carried out only once for each sample under the white light, though the counting time was different from sample to sample (Fig. 3). The experiments in Table 2 show that the rate of rhizoid formation increased with the time and reached a steady maximum rate after 16 hr or 24 hr, although Figure 3 indicates that the rate reached a plateau already after 8 hr. In the following experiments, therefore, number of rhizoid cells was counted after the sample was incubated for more than 16 hr. The dark incubation following illumination was designated as "postincubation" thereafter.

The material was cultured on a 12 hr - 12 hr light-dark cycle. In order to know whether or not the time of cutting in the light-dark cycle may influence rhizoid formation, the cultured filaments were cut eight times a day with 3 hr interval. The cut segments were preincubated, illuminated and postincubated following the ordinary experimental procedure. The result in Figure 4 indicates that there was no significant difference in the rhizoid formation among the specimens cut at various phases of the light-dark cycle. This suggests that no photoperiodic change was involved in the potency of the cell to form rhizoid. Also the absence of a diurnal rhythum in the dark period during preincubation is suggested by the fact that there was no periodic change in the rate of rhizoid formation in the material which was incubated in the dark for various hours (Fig. 2).

Phytochrome as a photoreceptor pigment

1. Effect of red light

In the above it has been made clear that light is indispensable for the rhizoid formation. A question arises then which kind of light is most effective for the rhizoid formation. An experiment using various colored lights (5.4×10^2 ergs cm^{-2} sec^{-1}, for 1 min) suggested that the light around 645 nm was most effective and that the light with a wave length shorter than 550 nm was only moderately effective (Table 3).

In order to confirm that red light was effective for the induction of the rhizoid formation, the dosage of red light (around 645 nm) was changed and the rate of rhizoid formation was investigated (Fig. 5). The light was given at 5.4 × 10^2 ergs cm^{-2} sec^{-1} for various periods of time. Fifteen sec irradiation of red light seemed to be enough for saturation of the effect. Therefore, the minimum energy of red light to saturate the effect was calculated to be 8.1 Kergs cm^{-2}.

2. Reversible effect of red and far-red light

The fact that red light stimulated the photomorphogenesis suggested a possibility that the photoreceptor might be phytochrome. Therefore it was examined whether or not the red light-induced rhizoid formation could be annulled by subsequent irradiation of far-red light (around 720 nm) given immediately after the red irradiation. The result indicated that far-red light annulled the inducing effect of red light. Then it was studied how much dosage of far-red light is enough for saturation of its inhibitory effect. The segments were exposed to far-red light at 4.6 Kergs cm^{-2} sec^{-1} for various periods of time after they were irradiated by red light at 5.4 × 10^2 ergs nm^{-2} sec^{-1} for 2 min. The rate of rhizoid formation decreased with the increase of the far-red dosage and the irradiation of 60 sec repressed formation of the rhizoid almost completely (Fig. 6). Thus, the minimum energy ncessary for a complete inhibition of the red light-induced rhizoid formation was estimated to be 2.8 × 10^2 Kergs cm^{-2}. But this amount of dosage was not always necessary for the complete inhibition. For the material exhibiting a low rate of rhizoid formation, only 40 sec irradiation of far-red light of the same intensity, is sufficient to inhibit completely the formation of the rhizoids.

In order to confirm the participation of phytochrome in the rhizoid formation, the photoreversibility between red and far-red light was studied further. The cell was exposed alternately to red light (1.1 Kergs cm^{-2} sec^{-1}) for 2 min and to far-red light (7.9 Kergs cm^{-2} sec^{-1}) for 2 min. The result (Table 4) shows clearly that the rate of rhizoid formation reached as high as about 50% when the final light of the cyclic irradiation was red, while the rate was nearly zero percent when the final light was far-red. That is, the red light-stimulated rhizoid formation was inhibited perfectly by subsequent far-red light. The inhibition was so complete that the rate of rhizoid formation after irradiation of far-red light in the cyclic treatments was as low as that of the far-red light control and the dark control. On the basis of this typical photoreversibility it is strongly suggested that the photoreceptor for the rhizoid formation in Spirogyra is phytochrome, or this photomorphogenetic reaction is mediated by phytochrome system.

3. Existence of phytochrome in Spirogyra cell

When the intact Spirogyra filaments were examined using differential spectrophotometry (3), phytochrome was detected. As shown in Figure 7, a difference absorbance between 650 nm and 710 nm gave a photoreversible response to alternating irradiations of red and far-red lights. The existence of phytochrome in the Spirogyra cell also supports the possibility that the photoreaction is controlled by phytochrome.

4. Escape reaction

In the above experiments the dark period between red and far-red irradiations was less than 5 sec. To clarify the relationship between the intervening darkness and the inhibitory effect of far-red light, the cell exposed to red light (1.1 Kergs cm^{-2} sec^{-1}, for 2 min) was subsequently irradiated by far-red light (7.9 Kergs cm^{-2} sec^{-1}, for 2 min) after various periods of dark interval. The result is represented in Figure 8. The cells gradually escaped from the inhibitory effect of far-red light with the increase in the dark interval. The half time for the escape reaction was obtained to be $2\frac{2}{3}$ hr. As a matter of course the escape reaction was completed within 16 hr, because rhizoid had been already formed at this time (Table 2, Fig. 3). When the dark-incubated cells were observed under dim green light 1 hr after the irradiation of red light, that is, immediately before the far-red irradiation, rhizoid was not yet formed. The subsequent far-red irradiation at this time did not inhibit the rhizoid formation perfectly and allowed to form it at a rate of 37%. This is interpreted in the following way. In some cells the photoreaction already went over a step at which the reaction became insensitive to far-red light, although the morphological differentiation of the cells was not detectable when they were exposed to far-red light. While in other cells the reaction proceeded slowly so that it did not reach the step yet and therefore it was interrupted by far-red irradiation.

5. Other pigments

Investigations for the existence of other pigment in the photoreaction system were also performed. First, it was examined whether or not blue or green light also repressed the rhizoid formation. The filaments exposed to red light (5.4×10^2 ergs cm^{-2} sec^{-1}, for 2 min) and subsequently to blue light (around 476 nm, 1.4 Kergs cm^{-2} sec^{-1}, for 2 min) or to green light (around 500 nm, 1.4 Kergs cm^{-2} sec^{-1}, for 2 min) formed rhizoids as frequently as those exposed to red light only (Table 5). This indicates that blue and green lights had no inhibitory action on the photomorphogenesis. As shown in Table 3 blue or green light alone was effective although they were less effective than red light. Second, an experiment was done where the material was first illuminated either with blue (5.4×10^2 ergs cm^{-2} sec^{-1}, for 2 min) or green (5.4×10^2 ergs cm^{-2} sec^{-1}, for 2 min) light and then with far-red (4.6 Kergs cm^{-2} sec^{-1}, for 2 min) light. As indicated in Table 6 the rhizoid formation induced by blue or green light was almost perfectly repressed, just as was the case in rhizoid formation induced by red light, by subsequent far-red light. Therefore the rhizoid inducing effect of blue or green light is considered to be caused by phytochrome, because any pigment having an absorption in far-red region and also participating in plant-photomorphogenesis has not yet been found except phytochrome.

Discussion

In lower plants several phytochrome-controlled photoreactions are known, such as chloroplast movement in Mesotaenium (4) and Mougeotia (5), oospore germination of Chara (6), spore germination (7), elongation of protonemata (8), cell division in gametophytes (9) of ferns, etc. However, participation of phytochrome in rhizoid formation has not been known.

Weihe (10) studied effects of various colored lights on rhizoid formation in Spirogyra fluviatilis. In his experiment the material was cultured for 10 days with various colored daylights transmitted through interference filters. It was shown that blue light (around 490 nm) promoted the rhizoid formation while red (around 700 nm) and green (around 570 nm) lights did not. The results obtained with blue and red lights seem to be not contradictory to those obtained in the present study, because high dosage of blue light may be capable of inducing the photomorphogenesis (cf. Table 6), and light of around 700 nm may be absorbed more by the P_{FR}-form of the phytochrome than by the P_R-form thus exhibiting an inhibitory action like far-red light used in the present study. But as for green light, the same promoting effect as that of blue light should be expected, since in the present experiment even brief irradiation with green light around 550 nm was effective (Table 3).

Hartmann (11) reported high energy reaction in lettuce seedlings. The action spectrum for inhibition of hypocotyl lengthening indicated that the light between 500 and 700 nm and that longer than 760 nm were ineffective to inhibit the lengthening. If a similar high energy reaction also exists in rhizoid formation of Spirogyra the result obtained with 10 days irradiation by Weihe may be reasonable.

Participation of blue light of low energy in photomorphogenesis has been reported, for example, on ferns for spore germination (7, 12, 13) and cell division in gametophytes (9). In these photoreactions blue light acts inhibitory for the action of red light, and has interaction with phytochrome. In Nostoc the development of motile trichomes from sheathed aseriated colonies was photocontrolled (14, 15). That is, red light (max. 640 nm) promoted colony breakage but green light (max. 520 nm) did not. The photomorphogenetic effects of either red or green light were reversible by irradiation with the other colored light. In rhizoid formation of Spirogyra such photoreactions are not involved.

The rhizoid formation was induced with a brief irradiation of red light of low intensities and the minimum energy to saturate the effect is estimated to be 8.1 Kergs cm^{-2}. This value is low and comparable to energies obtained in other phytochrome-dependent photoreactions. Rhizoid formation induced with red light was inhibited completely by subsequent far-red irradiation. This reversibility was maintained perfectly in repeated treatments with the two lights. The half time for the escape reaction was short. All these results indicate that rhizoid formation of Spirogyra is a typical phytochrome-mediated photoreaction.

The Spirogyra filament is a single array of cells. Each cell can form a rhizoid when it is isolated. In order to study the mode of action of phytochrome a photoreaction in a single cell may be more advantageous than that in a multicellular system, because in the former, interactions between cells need not be considered. The Spirogyra cell does not age as far as it is transplanted periodically. It can form a rhizoid a few hours after the illumination at any time. Thus rhizoid formation in the Spirogyra cell may be a very advantageous photocontrolled system for the study of phytochrome action.

Acknowledgements

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References

(1) Nagata, Y.: Rhizoid differentiation in *Spirogyra*. I. Basic features of rhizoid formation.

(2) Sussman, A. S.: Physiology of dormancy and germination in the propagules of cryptogamic plants. In *Encyclopedia of Plant Physiology* XV. Edited by W. Ruhland. p. 933-1025. Springer-Verlag Inc., Heidelberg, 1965.

(3) Butler, W. L., K. H. Norris, H. W. Siegelman and S. B. Hendricks: Detection, assay and preliminary purification of the pigment controlling photoresponsive development of plants. *Proc. N. A. S.* 45: 1705-1708 (1959).

(4) Haupt, W. and R. Thiele: Chloroplastenbewegung bei *Mesotaenium*. *Planta* 56: 388-401 (1961).

(5) Haupt, W.: Die Chloroplastendrehung bei *Mougeotia*. I. Über den Quantitativen und Qualitativen Lichtbedarf der Schwachlichtbewegung. *Planta* 53: 484-501 (1959).

(6) Takatori, S. and K. Imahori: Lightreactions in the control of oospore germination of *Chara delicatula*. *Phycologia* 10: 221-227 (1971).

(7) Sugai, M. and M. Furuya: Photomorphogenesis in *Pteris vittata*. I. Phytochrome-mediated spore germination and blue light interaction. *Plant & Cell Physiol.* 8: 737-748 (1967).

(8) Miller, J. H. and P. M. Miller: Effects of red and far-red illumination on the elongation of fern protonemata and rhizoids. *Plant & Cell Physiol.* 4: 65-72 (1963).

(9) Wada, M. and M. Furuya: Phytochrome action on timing of cell division in *Adiantum* gametophytes. *Plant*

Physiol. 49: 110-113 (1972).

(10) Weihe, K. von: Über die Rhizoidbildung und die Rhizoidzelle bei *Spirogyra*. *Zeitschr. Bot.* 40: 231-249 (1952).

(11) Hartmann, K. M.: Ein Wirkungsspektrum der Photomorphogenese unter Hochenergiebedingungen und seine Interpretation auf der Basis des Phytochroms (Hypokotylwachstumshemmung bei *Lactuca sativa* L.). *Z. Naturforschg.* 22b: 1172-1175 (1967).

(12) Sugai, M. and M. Furuya: Photomorphogenesis in *Pteris vittata*. II. Recovery from blue light-induced inhibition of spore germination. *Plant & Cell Physiol.* 9: 671-680 (1968).

(13) Sugai, M.: Photomorphogenesis in *Pteris vittata*. IV. Action spectra for inhibition of phytochrome-dependent spore germination. *Plant & Cell Physiol.* 12: 103- 109 (1971).

(14) Lazaroff, N. and J. A. Schiff: Action spectrum for developmental photo-induction of the blue-green alga *Nostoc muscorum*. *Science* 137: 603-604 (1962).

(15) Robinson, B. L. and J. H. Miller: Photomorphogenesis in the blue-green alga *Nostoc commune* 584. *Physiologia Plantarum* 23: 461-472 (1970).

Table 1. Rhizoid formation under various periods of illumination.

Length of Illumination	Rate of Rhizoid Formation (%)
5 min	38
10 min	43
30 min	54
1 hr	52
8 hr	53

The short filaments preincubated in the dark for 24 hr were illuminated for various periods of time at 1,000 lux, then postincubated. Each value represents the mean of 6 separate experiments.

Table 2. Rhizoid formation after different periods of postincubation.

Experiment	Rate of Rhizoid Formation (%)								
	Length of Postincubation (hr)								
	3 →	6 →	8 →	16 →	24 →	31 →	39 →	48 →	64
1	22	42	46		46	44			48
2	25	42	44		42	44			50
3	32	59	64		59			59	
4	19	37	41		50			50	
5				54	52				54
6				57	57		57		57
7				77	77		77		77

The preincubated segments were illuminated at 1,000 lux, for 25 min, then postincubated. The number of rhizoid cells were counted under dim green light at different periods of time after the onset of postincubation.

Table 3. Effect of various colored lights on rhizoid formation.

Peak Wave Length of Colored Lights (nm)	Rate of Rhizoid Formation (%)
645	69
610	54
550	29
500	28
476	24
440	22

Preincubated segments were irradiated with various colored lights (540 ergs cm^{-2} sec^{-1}) for 1 min, then postincubated. Each value represents the mean of 4 separate experiments.

Table 4. Reversibility of red light effect on rhizoid formation by far-red light.

	Rate of Rhizoid Formation (%)				
	Experiment 1	2	3	4	Average
D	3.1	0	0	0	0.8
FR	0	0	0	0	0
R	38	70	25	63	49
R/FR	0	0	0	0	0
R/FR/R	30	76	38	44	47
R/FR/R/FR	0	0	7.4	0	1.9
R/FR/R/FR/R	50	69	41	52	53
R/FR/R/FR/R/FR	0	0	0	0	0
R/FR/R/FR/R/FR/R	54	31	34	58	44
R/FR/R/FR/R/FR/R/FR	0	0	0	0	0

Preincubated segments were irradiated with 1.1 Kergs cm^{-2} sec^{-1} red light (R) for 2 min and/or with 7.9 Kergs cm^{-2} sec^{-1} far-red light (FR) for 2 min, and then post-incubated. This alternate irradiation of red light and far-red light was repeated up to 4 times. D; the dark control treated with the same way as red light irradiation except that red light was intercepted by a black filter to reach the segments.

Table 5. Rhizoid formation with blue or green light given subsequently after red light irradiation.

	Rate of Rhizoid Formation (%)
R	51
R/B	53
R/G	53

Preincubated segments were irradiated with red light (R) (5.4×10^2 ergs cm^{-2} sec^{-1}) for 2 min with or without irradiation of blue light (B) or green light (G) (both, 1.4 Kergs cm^{-2} sec^{-1}) for 2 min, then post-incubated. Each value represents the mean of 4 separate experiments.

Table 6. Inhibitory effect of far-red light on blue or green light-stimulated rhizoid formation.

	Rate of Rhizoid Formation (%)
R	52
R/F	2.5
B	32
B/F	1.0
G	15
G/F	1.0

Preincubated short segments were irradiated with 540 ergs cm^{-2} sec^{-1} red light (R), blue light (B) or green light (G) for 2 min with or without following irradiation of 4.6 Kergs cm^{-2} sec^{-1} far-red light (F) for 2 min, and then postincubated. Each value represents the mean of 4 separate experiments.

Legends for Figures

Fig. 1. Transmission bands obtained with various combinations of filters used. The number given at each curve represents the peak wavelength. B, blue light; G, green light; R, red light; FR, far-red light.

Fig. 2. Relation of preincubation time in darkness with rhizoid formation. Short fragments of filaments preincubated for various periods were illuminated at 1,000 lux for 30 min, then postincubated for 16 to 24 hr.

Fig. 3. Rate of rhizoid formation after various periods of postincubation. For 24 hr preincubated filaments were illuminated at 1,000 lux, for 20 min, then postincubated for various periods. Each point represents the mean of 2 separate experiments.

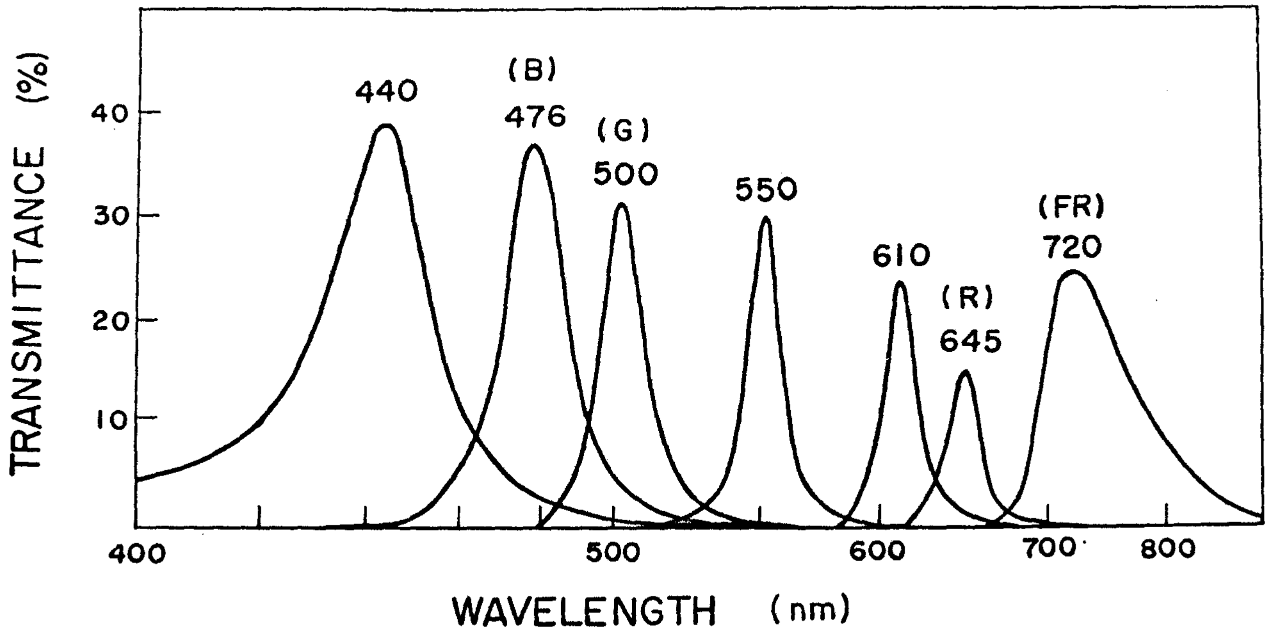
Fig. 4. Rhizoid formation of filaments cut at various stages of light-dark cycle. After the cultured filaments were cut at 3, 6, 9, 12, 15, 18, 21 and 24 o'clock, they were preincubated, illuminated (1,000 lux for 40 min) and postincubated.

Fig. 5. Rhizoid formation versus dosage of red light irradiation. Preincubated filaments were irradiated with red light (5.4×10^2 ergs cm^{-2} sec^{-1}) for various periods, then postincubated. Each plot represents the mean of 5 separate experiments.

Fig. 6. Rhizoid formation versus dosage of far-red light irradiation. Preincubated segments were irradiated with red light (5.4 × 10^2 ergs cm^{-2} sec^{-1}) for 2 min and thereafter with far-red light (4.6 Kergs cm^{-2} sec^{-1}) for various periods before they were postincubated. Each plot represents the mean of 4 separate experiments.

Fig. 7. Difference absorbance of Spirogyra filaments between 650 nm and 710 nm after alternate irradiation of red and far-red lights.

Fig. 8. Rhizoid formation escaping the inhibition of far-red light. Preincubated segments were irradiated with red light (1.1 Kergs cm^{-2} sec^{-1}) for 2 min and incubated in the dark for various periods before the irradiation with far-red light (7.9 Kergs cm^{-2} sec^{-1}) for 2 min. After far-red irradiation the segments were postincubated. Each plot represents the mean of 4 separate experiments.



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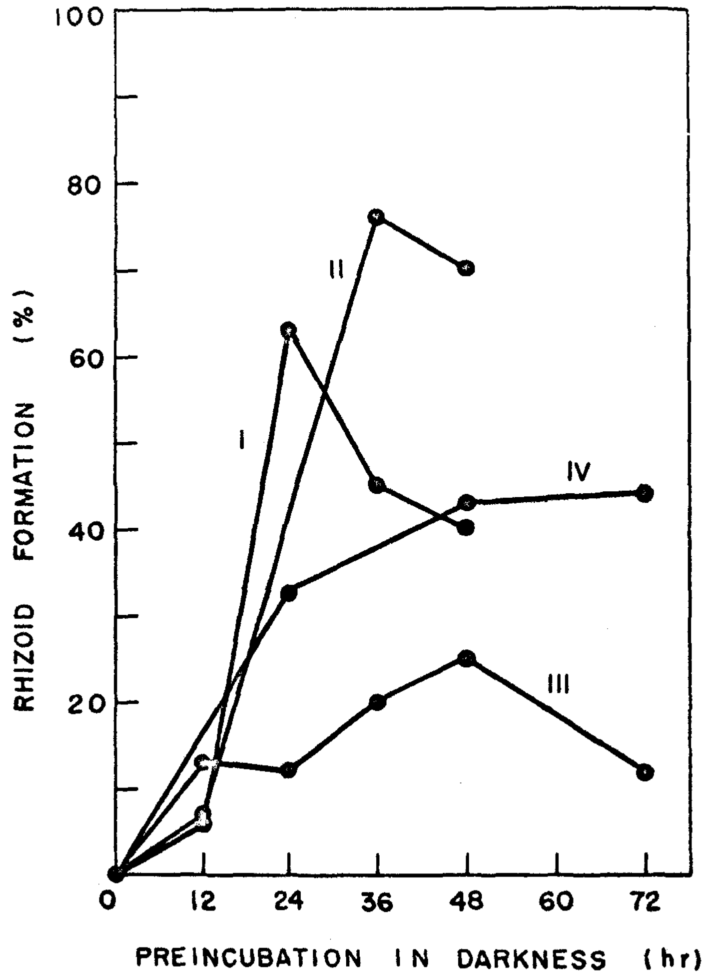

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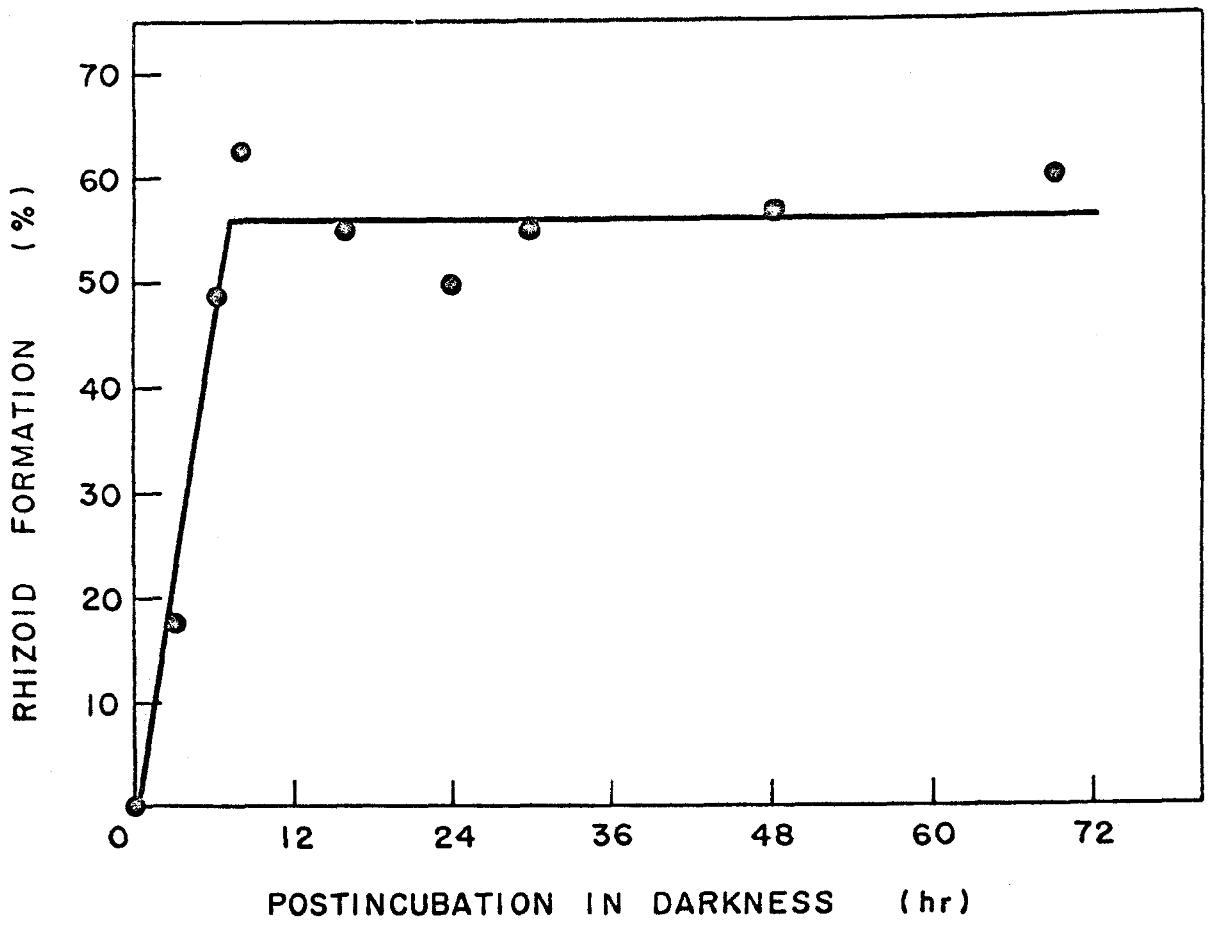

Fig. 3. Rate of rhizoid formation after various periods of postincubation. For 24 hr preincubated filaments were illuminated at 1,000 lux, for 20 min, then postincubated for various periods. Each point represents the mean of 2 separate experiments.

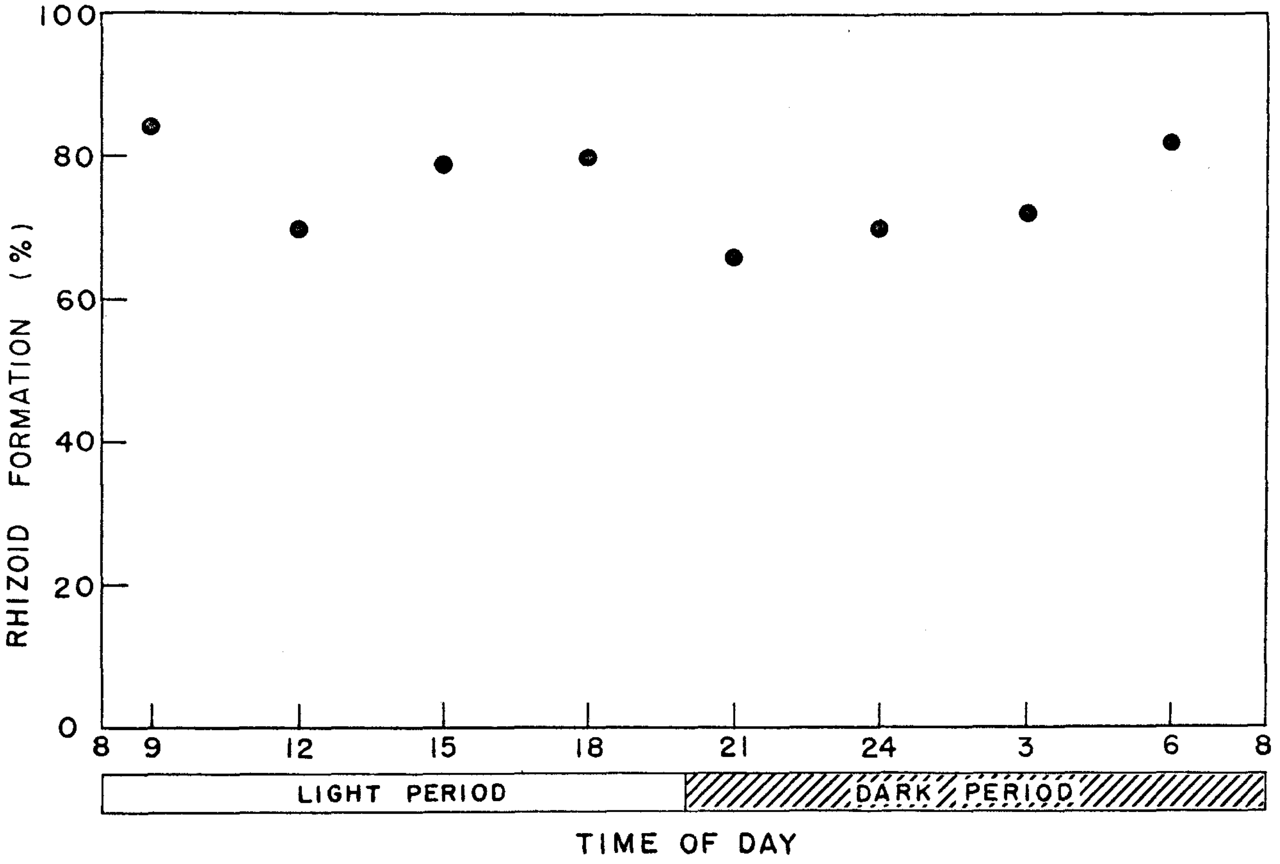

Fig. 4. Rhizoid formation of filaments cut at various stages of light-dark cycle. After the cultured filaments were cut at 3, 6, 9, 12, 15, 18, 21 and 24 o'clock, they were preincubated, illuminated (1,000 lux for 40 min) and postincubated.

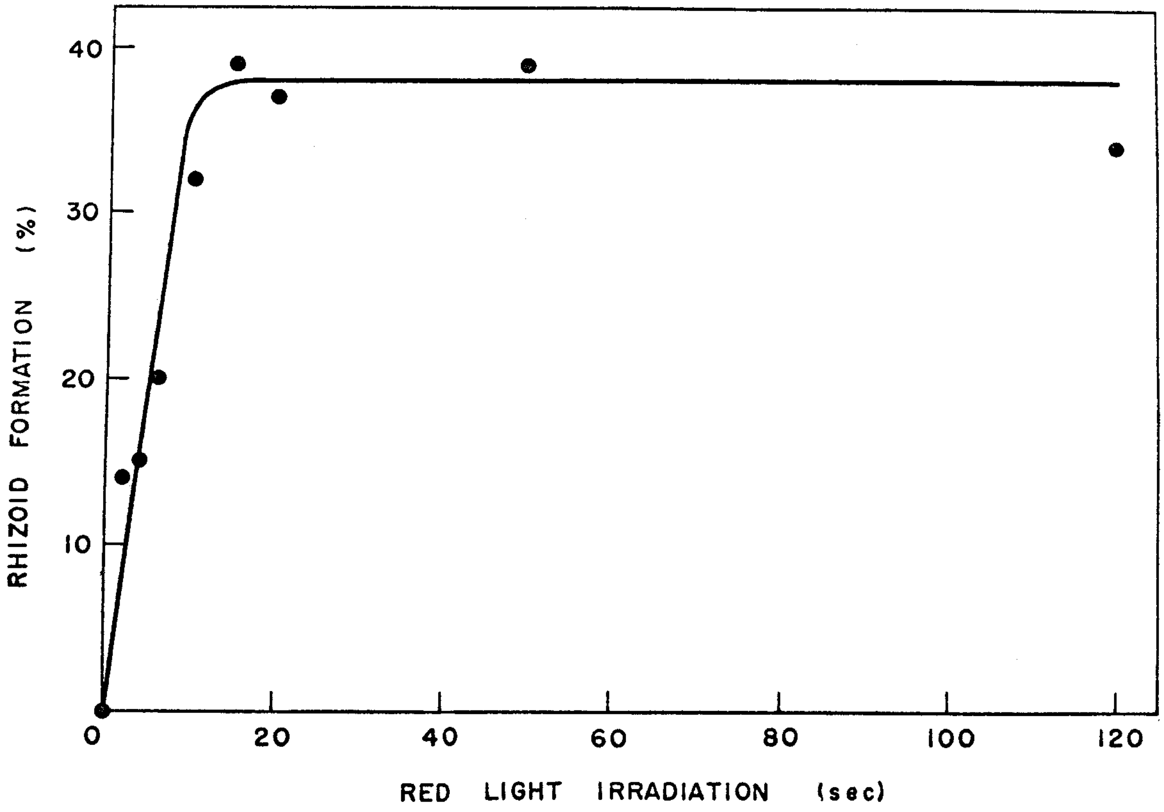

Fig. 5. Rhizoid formation versus dosage of red light irradiation. Preincubated filaments were irradiated with red light (5.4 × 10^2 ergs cm^{-2} sec^{-1}) for various periods, then postincubated. Each plot represents the mean of 5 separate experiments.

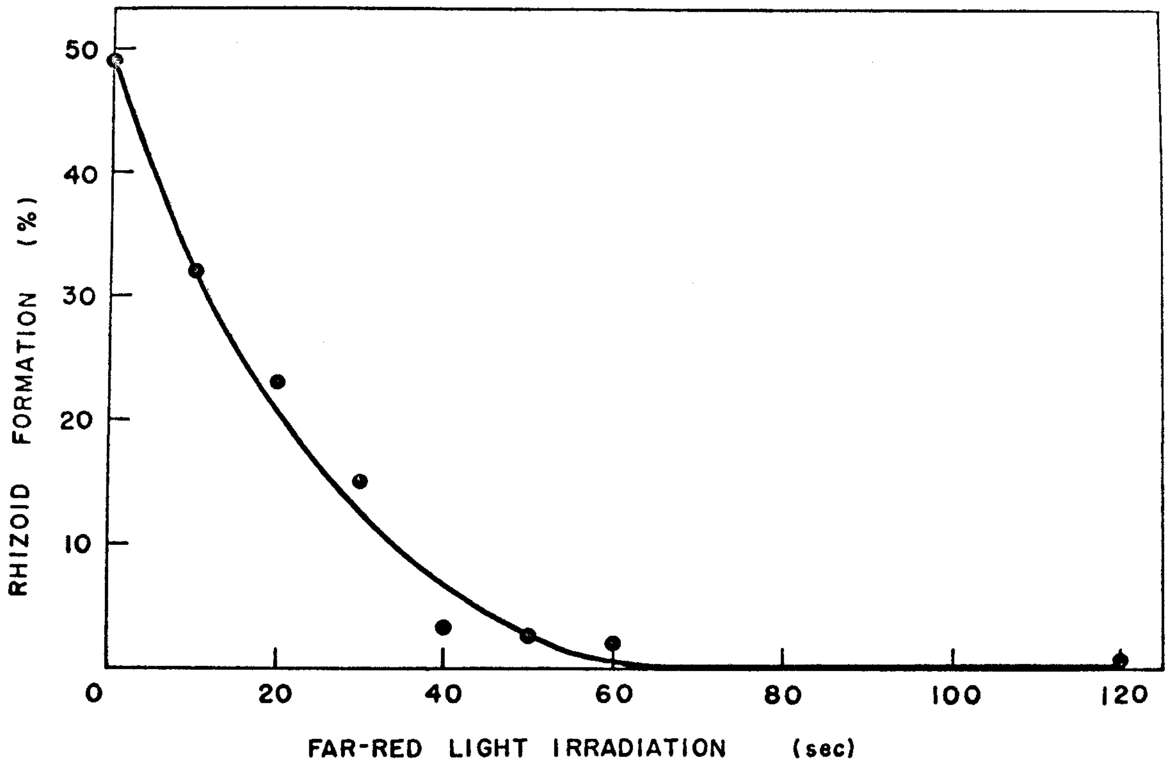

Fig. 6. Rhizoid formation versus dosage of far-red light irradiation. Preincubated segments were irradiated with red light (5.4 × 10^2ergs cm^{-2} sec^{-1}) for 2 min and thereafter with far-red light (4.6 Kergs cm^{-2} sec^{-1}) for various periods before they were postincubated. Each plot represents the mean of 4 separate experiments.

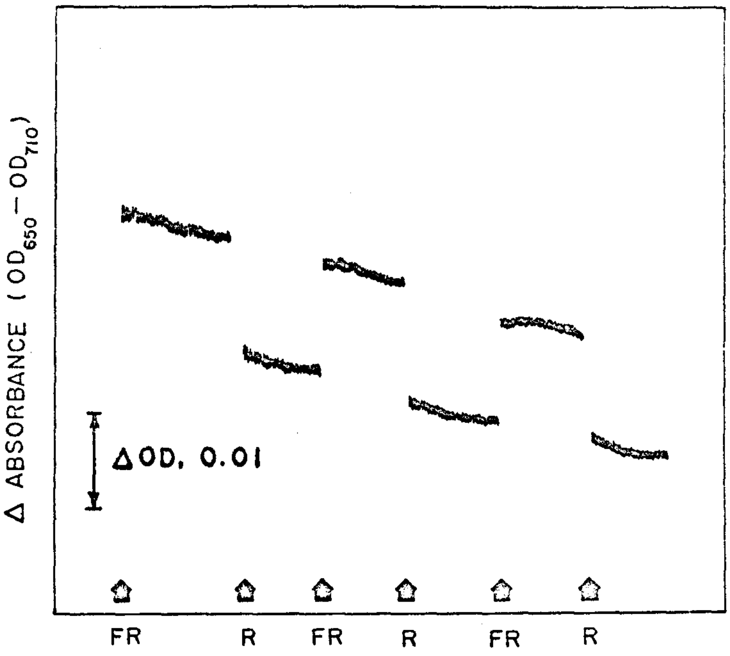

Fig. 7. Difference absorbance of Spirogyra filaments between 650 nm and 710 nm after alternate irradiation of red and far-red lights.

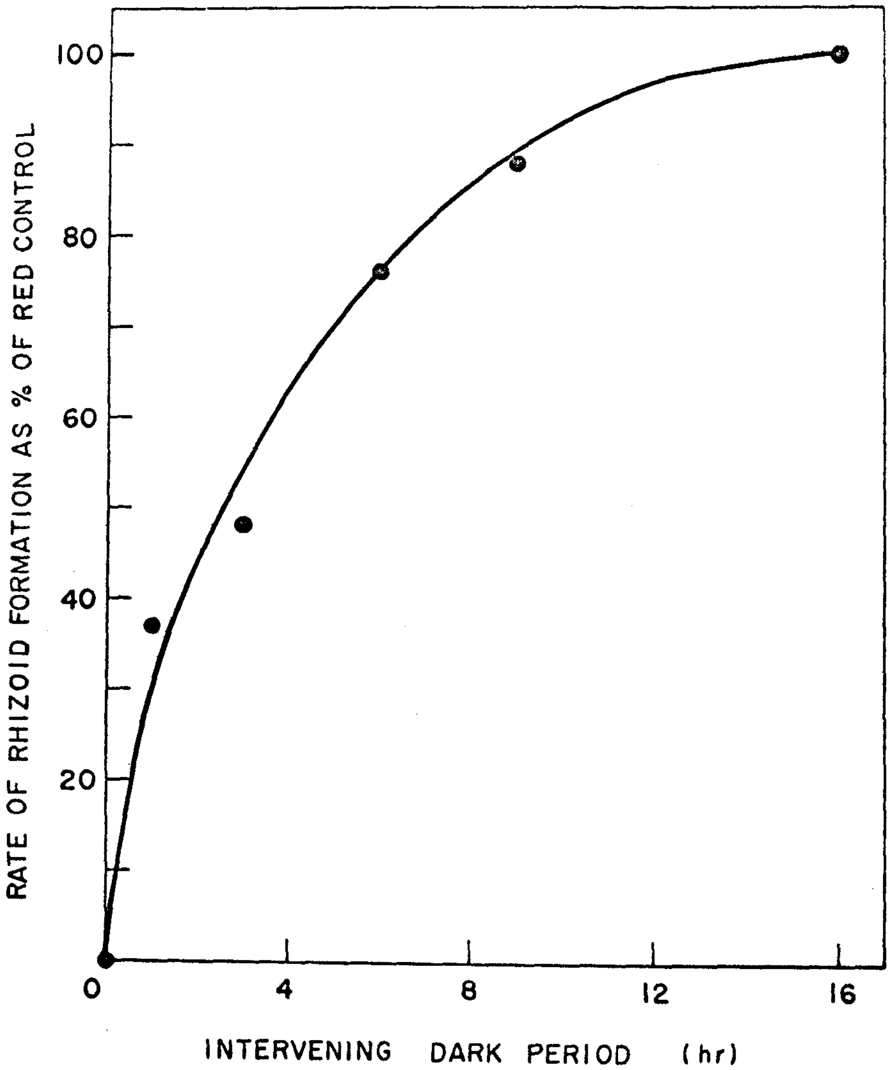

Fig. 8. Rhizoid formation escaping the inhibition of far-red light. Preincubated segments were irradiated with red light (1.1 Kergs cm^{-2} sec^{-1}) for 2 min and incubated in the dark for various periods before the irradiation with far-red light (7.9 Kergs cm^{-2} sec^{-1}) for 2 min. After far-red irradiation the segments were postincubated. Each plot represents the mean of 4 separate experiments.