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Effects of microbeam light on growth and phototropism of *Pilobolus crystallinus* sporangiophores

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A small blue-light beam (50 μm in diam) was used to examine light-growth response and phototropism in *Pilobolus crystallinus* sporangiophores. Continuous irradiation by microbeam of a region 100–150 μm from the apex promoted the growth of a dark-adapted sporangiophore for about 15 min after a lag period of 1–2 min. After the promotion, the growth rate fell below that before the irradiation. Irradiation of the apex of sporangiophore slightly promoted the growth but strongly inhibited the growth after the promotion. A smaller light beam (10 μm in diam) applied continuously at grazing incidence along one side of the sporangiophore caused bending toward the shaded side, implying that the irradiated side grew more rapidly than the shaded side and that the lens effect is involved in the phototropism of young sporangiophores of *P. crystallinus*. The involvement of the lens effect was confirmed by the fact that a carotenoid-less mutant was 1.5–2 times more sensitive to unilateral blue light than the wild type, probably because of a smaller intracellular light attenuation during passage through the mutant cell.

Key Words——blue light; light growth response; microbeam irradiation; phototropism; *Pilobolus crystallinus*.

Phototropic bending is accomplished either by a difference in growth rate between irradiated and shaded sides or by a change in the direction of apical growth, i.e., displacement of the growing region to the irradiated side. The former is the case for phototropic bending of higher plants (Firn, 1986), and the latter for that of fern protonema (Etzold, 1965) or algae, *Vaucheria* (Kataoka, 1975).

Phototropic response has also been examined in coprophilous fungi *Pilobolus crystallinus* (Wiggers) Tode and *Pilobolus kleinii* van Tiegh. Mature sporangiophores of *Pilobolus* bend toward light through differential growth between the irradiated and shaded sides. In young sporangiophores, however, the bending mechanism is complicated. Buller (1934) reported that, in *P. crystallinus*, light focused by the lens effect causes differential growth between the irradiated and shaded sides of a sporangiophore, which results in a positive tropism; but in *P. kleinii*, a lens effect is not involved because the incident light is attenuated by carotenoids during passage through the cell. In the latter case, light should be considered to suppress the growth of the irradiated side causing a positive curvature. Page (1962) reported that, in both *P. crystallinus* and *P. kleinii*, unilateral light changes the direction of apical growth to the irradiated side causing a positive curvature without involvement of a lens effect. Later, however, Page and Curry (1966) found that if a small light beam grazed along one side of the sporangiophore of *P. kleinii*, the sporangiophore bent toward the other side. This indicates that tropic bending of *P. kleinii* is accomplished by differential growth. In this study, we examined the light-growth response and phototropic response by using a small blue-light beam to examine the mechanism of phototropic bending in *P. crystallinus* sporangiophores.

Materials and Methods

Pilobolus crystallinus, strain IFO8561, obtained from the Institute for Fermentation, Osaka, was cultured on MYC agar (1% malt extract, 0.2% yeast extract, 0.2% casamino acids and 1.2% agar) at 23±1°C under continuous white light of about 8 Wm^{-2} (fluorescent tubes, FL20SSD/18; Mitsubishi, Tokyo, Japan). Four or five days after inoculation, agar blocks each with many trophocysts were cut out from the culture plate and placed in a small glass case (75×26×3 mm) or a screw-capped glass tube (12×125 mm) (Kubo and Mihara 1988). These containers were placed in darkness at 23±1°C for about 24 h to induce sporangiophore formation from the trophocysts. When many young sporangiophores were formed, the containers were transferred onto the stage of a microscope under a red safety light. Sporangiophores were placed horizontally because *Pilobolus* sporangiophores show no gravitropism, and a microbeam was shone continuously from the bottom. A microbeam of blue light (450 nm) obtained from a microspectrophotometer (Model MMSP; Olympus Optical Co. Ltd., Tokyo) was used for irradiation.

In the experiment in Fig. 4, blue light (450 nm) was obtained from a Xenon lamp equipped with a grating

monochromator (Jasco CT-25; Japan Spectroscopic Co., Tokyo) and applied continuously from one side. The fluence rate was attenuated by inserting ND filters between the light source and the experimental container.

For isolation of a carotenoid-less mutant, spores were treated with 1-methyl-3-nitro-1-nitrosoguanidin (200 μg/ml) for about 10 min and streaked on MYC agar medium. Carotenoid-less mutant was selected with the naked eye.

Growth of the sporangiophore was measured every 1 min under a red safety light by using a micrometer. To measure the bending rate and lag period for phototropic curvature, a photograph was taken using red safety light every 10 min.

Results

The effects of irradiation of the regions 0-50, 50-100 and 100-150 μm away from the apex on the growth of the sporangiophore were compared. A beam of blue light (50 μm in diam), which is slightly larger than the diameter of sporangiophore, was applied continuously to the sporangiophore, and the length of the sporangiophore was measured every 1 min. The beam was unilaterally applied from the bottom because the sporangiophore did not bend toward the light significantly during the experimental period.

Figure 1 shows the relation between growth rate before the irradiation and percentages of increase or decrease in growth rate after the irradiation. Growth rates of sporangiophores were ranged from 2.6 to 9.0 μm/min but there was no clear correlation between the growth rate before the irradiation and the magnitude

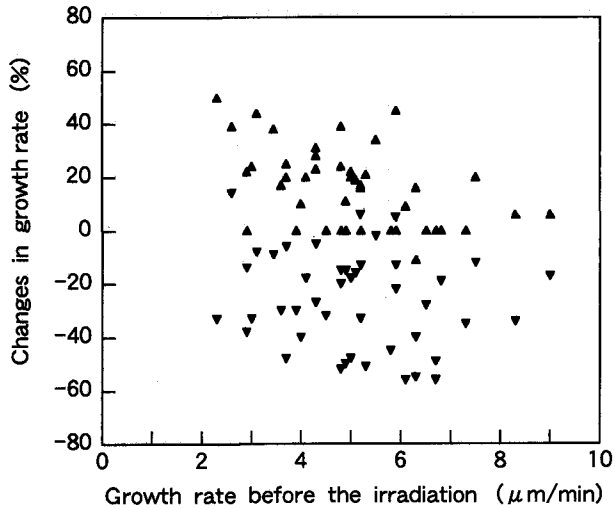

Fig. 1. Relation between the growth rate of sporangiophores before irradiation and the relative change in growth rate after the irradiation. Growth rates were determined 0-10 min before the irradiation (A), 3-20 min after the start of irradiation (B) and 20-25 min after the start of irradiation (C).
(▲): percentage change in growth rate (B-A)/A.
(▼): percentage change in growth rate (C-A)/A.

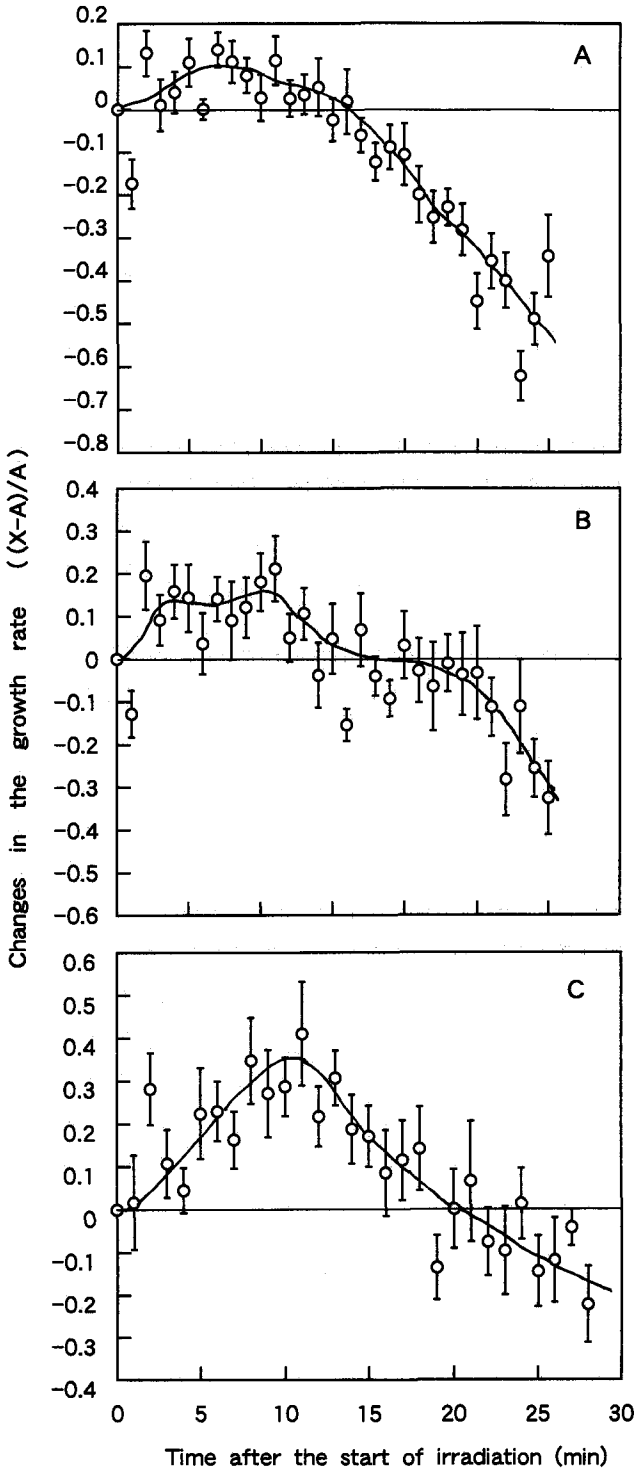

Fig. 2. Effect of blue light (2.84 μmol/m²s) on the growth of *Pilobolus* sporangiophores. A beam of blue light (50 μm in diam) was applied to sporangiophores 0-50 (A), 50-100 (B) or 100-150 μm (C) from the apex. Growth (increase in length) of sporangiophores was measured every 1 min under a red safety light. The values (X-A)/A (X: growth rate at given time after the start of irradiation; A: growth rate before the irradiation) were plotted against time (min). Each point represents the average of 11-19 sporangiophores. Bars=SE.

of changes in growth rate after the irradiation.

Irradiation of the apex slightly promoted the growth of the sporangiophore but strongly lowered the growth rate after a lag period of 15-20 min (Fig. 2A). Irradiation of the region 100-150 μm from the apex accelerated the growth with a lag period of 1-2 min, and the accelerated growth rate was maintained for about 15 min, followed by a reduction in the growth rate (Fig. 2C). Irradiation of the region 50-100 μm from the apex had an intermediate effect between the above two (Fig. 2B).

Next, the photoreceptive zone for phototropism was examined. A microbeam of blue light (10 μm in diam) was applied at grazing incidence along one side of the sporangiophore as shown in Fig. 3. The irradiation point was kept at nearly the same distance from the apex during the experimental period. The sporangiophore bent away from the irradiation point. This means that the irradiated side grew more rapidly than the other side. Irradiation of the point 50 μm from the apex was highly effective for phototropism, but irradiation of the point 300 μm from the apex had only a slight effect.

Figure 3 shows that the irradiated side of sporangiophore grew faster than the other side. This result suggested that a lens effect was involved in phototropism of young sporangiophores of *P. crystallinus*. To verify this, the fluence-response curves on the phototropic response in a carotenoid-less mutant were compared with those in a wild type strain. In the mutant, the shaded side receives stronger light than it does in the wild type, because light is attenuated less than in the wild type during its passage across the cell. Figure 4 shows the effect of unilateral blue light on the fluence-response curve for bending rate and lag period for the bending in both the carotenoid-less mutant and the wild type. In both strains, the bending rates increased with the increase in fluence rate and reached a maximum at about 20 pmol/m^{2}s. The lag period for phototropism was 20-30 min at a fluence rate lower than 10^4 pmol/m^2 s in both the mutant and wild type, and it was prolonged with the increase in fluence rate beyond this value. For both bending rate and lag period of phototropism, however, the mutant was about 1.5-2 times more sensitive to light than the wild type, which suggests that the

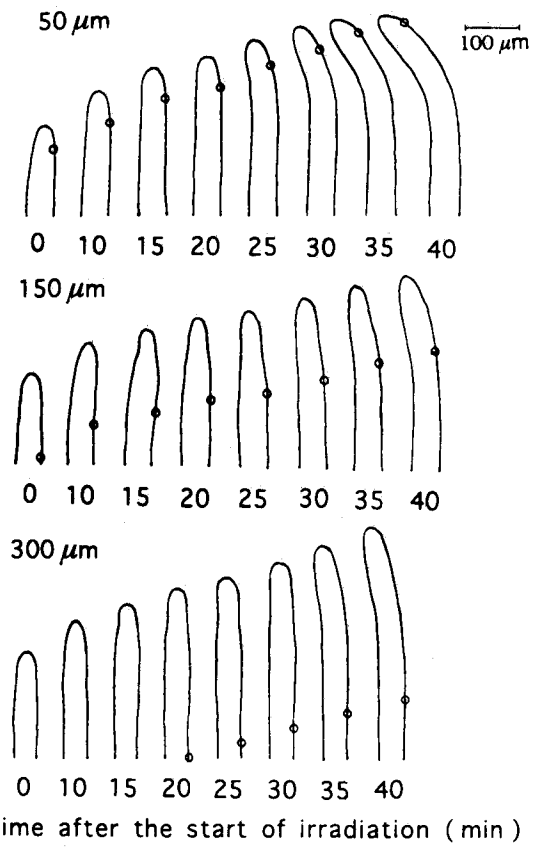

Fig. 3. Curvature of *Pilobolus* sporangiophores caused by microbeam irradiation. A microbeam of blue light (10 μm in diam) was applied at grazing incidence along one side of the sporangiophore, keeping the point of irradiation at 50 (top), 150 (middle) and 300 μm (bottom) from the apex during the irradiation period. Figures were traced from photographs taken at the indicated times.

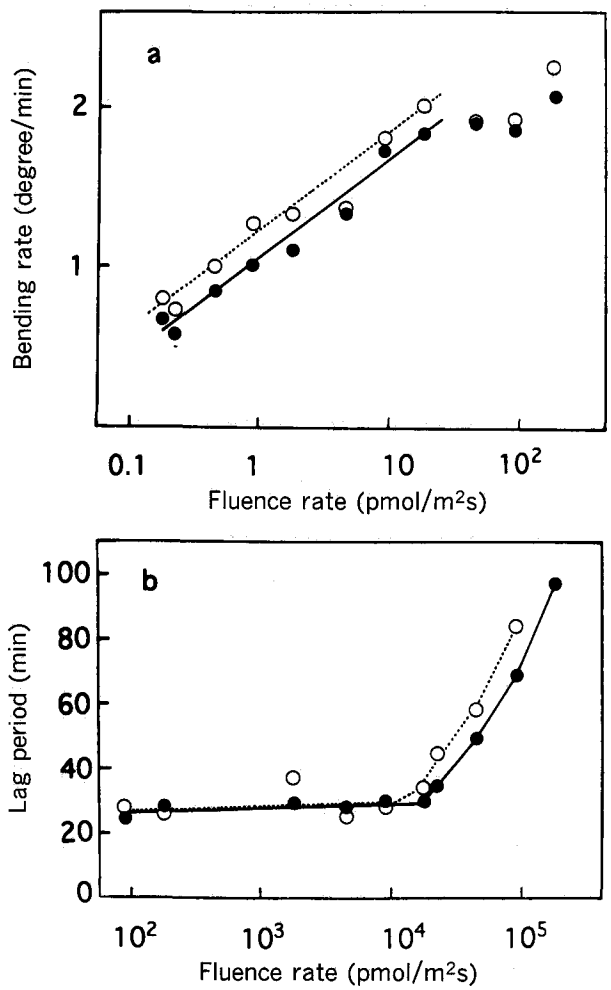

Fig. 4. Effects of fluence rate of unilateral blue light on the rate and the lag period of phototropic bending in the carotenoid-less mutant and the wild type of *Pilobolus*.
a: phototropic bending rate; wild type (●), mutant (○).
b: lag period for bending; wild type (●), mutant (○).

lens effect is involved in phototropism of young sporangiophores of *P. crystallinus*.

Discussion

In young sporangiophores of *P. crystallinus*, phototropic bending is accomplished by the differential growth between the irradiated side and the shaded side. Figure 3 clearly shows that the side of sporangiophore that received stronger light grows faster than the other side. Furthermore, Fig. 4 shows that the lens effect is involved in phototropism. These results support the observations reported by Buller (1934) in *P. crystallinus* and by Page and Curry (1966) in *P. kleinii* that light focused on the shaded side of a sporangiophore causes differential growth between the irradiated and the shaded sides, which results in a positive tropism.

Growth of the sporangiophore in *P. crystallinus* was accelerated by a step-up irradiation of blue light. The acceleration started after a 1-2 min lag period and lasted for 15 min. In *Phycomyces*, growth of the sporangiophore was promoted by a single pulsed light or step-up of the fluence rate (Bergman et al., 1969). The growth was promoted for 4-6 min with a 3-min lag period and the growth promotion was followed by growth suppression. The duration of growth promotion was longer in *Pilobolus* than *Phycomyces*, probably because the irradiation conditions were different. That is, sporangiophores were irradiated continuously in the present experiments but with pulse light in the experiments with *Phycomyces.*

Growth of *Pilobolus* sporangiophores was suppressed after the growth promotion. However, the suppression seems to be of a different nature from that in *Phycomyces,* which seems to be an aftereffect of the growth promotion. In *Pilobolus*, the growth suppression is considered to be a different photoreaction from the growth promotion because the most light-sensitive zone for growth promotion was 100-150 μm from the apex, while that for growth suppression was the apex (Fig. 2).

As mentioned above, phototropic bending is accomplished by growth promotion on the side receiving stronger light. However, the growth promotion involved in phototropic bending (Fig. 3) may differ from the light-growth promotion reaction that we observed in the microbeam experiment (Fig. 2). Irradiation of the region 100-150 μm from the apex strongly promoted the growth with a lag period of only 1-2 min (Fig. 2C), while irradiation of the apex or near the apex (50 μm) strongly suppressed the growth with a lag period of about 15-20 min (Fig. 2A). Phototropic bending, however, occurred exclusively when the area near the apex was irradiated but not the basal region (Fig. 3). In addition, the bending reaction appeared 20 min after irradiation. Thus, the lag period and the photosensitive zone for growth promotion differed from those for phototropic bending, implying that the growth promotion response observed here may be independent of the phototropic response. Iino and Schäfer (1984) also suggested in *Phycomyces* that the primary mechanisms of phototropic response and light-growth response are distinct.

On the other hand, the lag period and photosensitive zone for phototropic response (Fig. 3) are similar to those for growth suppression (Fig. 2). These results raise the possibility that bending is induced by growth suppression on the irradiated side of sporangiophore. As mentioned above, however, phototropic bending is accomplished by growth promotion on the side receiving stronger light. That is, we have to assume that some photoreactions other than the suppression of growth presented here are involved in phototropic response. Thus, it is suggested that the sporangiophore of *P. crystallinus* shows three different photoresponses, i.e., light-induced growth promotion, light-induced growth suppression and phototropism.

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