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Phototropism in Phycomyces as Investigated by Focused Laser Radiation

Abstract. *The phototropic response and distribution of photopigment in the sporangiophore of* Phycomyces *was investigated with a microillumination pattern. The data support the hypothesis that phototropism results from greater stimulation of growth in regions of more intense illumination and indicate that the photoreceptor extends to the outer wall of the sporangiophore.*

The single-celled sporangiophore of the fungus *Phycomyces blakesleeanus* shows a positive tropic response to visible light (*1*, *2*). The focusing properties of the transparent cylindrical sporangiophore suggest that phototropism may be caused by increased growth in the region of most intense illumination (*2*–*4*); but no direct proof of this hypothesis exists. In addition, the location of the photoreceptor is unknown.

We report here the use of a microillumination pattern as a stimulus of the phototropic response in order to test the above hypothesis and to study the distribution of photopigment. A 488-nm laser beam was imaged through the sporangiophore in the shape of a thin plane parallel to the long axis of the sporangiophore (Fig. 1). The distance, *f*, of the plane of illumination from the axis could be varied. The components of the tropic responses in both the *xz* and *yz* planes were measured after a stimulus of 1 minute of laser illumination. The response in the *xz* plane (Fig. 2) began at 3 to 4 minutes and continued until 13 minutes. The tropic angle, ϕ, was taken as the change in inclination of the sporangiophore in the *xz* plane between 2 and 12 minutes as measured on the edge of the photograph nearest to the sporangium. The bend is clearly away from the side illuminated (Figs. 2 and 3A); the magnitude of the response depends on *f* (Fig. 3B). There was also a smaller but appreciable component of the tropic response in the *yz* plane. However, there was considerable scatter in these data and no apparent correlation in the sign or magnitude of the response with the position of illumination, which indicated that the tropism in the *yz* plane was random. Thus, the principal phototropic response is in the plane perpendicular to the direction of incidence. The receptor mechanism cannot sense the direction of incidence but responds to the differential illumination. Our results support the hypothesis that phototropism is caused by enhanced growth in the more intensely illuminated regions.

The appreciable tropic response stimulated by illumination near the outer wall indicates that some photoreceptor must extend to this region of the sporangiophore. To evaluate more specific distributions of photopigment, we

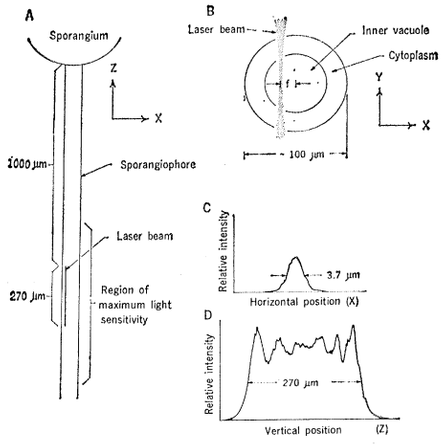

Fig. 1. (A) Schematic diagram of the sporangiophore and sporangium indicating the laser image as viewed along the direction of incidence. (B) Cross section of the sporangiophore. The path of the laser beam is shown assuming no scattering. Measurements of light scattering within the sporangiophore would better define the light distribution. (C) An experimental trace of the laser-beam intensity measured at the beam waist in the *x* direction. (D) An experimental trace of the laser beam intensity as measured along the sporangiophore axis (*z*).

constructed a simple model of the photoresponse. We assumed that any pigment in the light path is stimulated to produce a "growth factor" which diffuses randomly (in two dimensions for simplicity of calculation) until it reaches the outer wall (*5*). The inner membrane was treated as an impermeable reflecting boundary. The growth of each wall segment was assumed to be proportional to the amount of "growth factor" reaching that point and may be calculated by computer. The wall is elastic (*6*) and will bend to balance the internal forces set up by the uneven growth. The tropic angle was determined by a least-squares fit with the growth distribution.

Three hypothetical photoreceptor distributions were considered (Fig. 3B). The predicted tropic response for the pigment localized near the inner membrane is most clearly in disagreement with experiment. The assumption of a receptor near the outer wall also yields a large discrepancy between experiment and theory. Assignment of the photopigment to the cytoplasm or to both the inner and outer walls (curve not shown) gives predicted plots of tropic response which more closely correspond to our observations. The results indicate that the pigment cannot be confined to the region of the inner membrane and is probably not confined to the outer wall.

The laser, employed because of the collimation property of its radiation, was a continuous-wave argon laser at 488 nm operated in its lowest order spatial mode (T_{00q}) (*7*). The light, travelling in the *y* direction, was focused in the *x* direction by a cylindrical lens (2.5-cm focal length) forming a vertical line which was limited to 270 μm in length (along *z*) by a slit (Fig. 1, A and B). The width of the beam at the focus (Fig. 1C) was caused by diffraction associated with the 2-mm diameter of the beam at the lens. This configuration (*f*/12.5) was optimum for providing a narrow beam at the focus while avoiding large variations in beam width caused by focusing as the light traversed the 100 μm thickness of the sample. To obtain a nearly rectangular distribution of intensity in the *z* direction, Fresnel diffraction was minimized by placing the slits as close to the sample as was practical (*8*).

An inverted sporangiophore (Stage IVb) growing at a rate of 41 ± 9 μm/min in a shell vial of potato agar was held in a micromanipulator (*9*). The sporangiophore was immersed in a

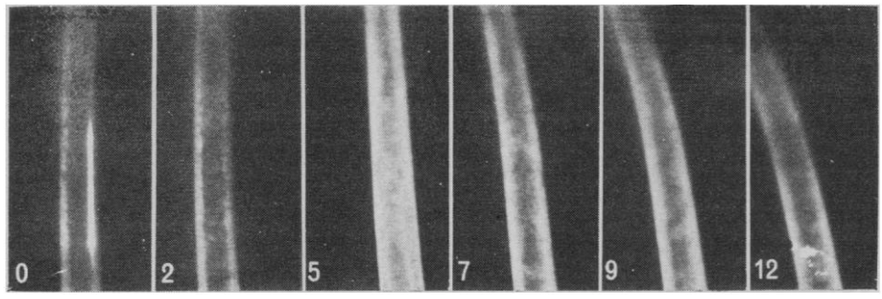

Fig. 2. The tropic response, ϕ, of the sporangiophore after a 1-minute stimulation with laser radiation. Time is measured from the onset of the stimulus. An image of the laser beam can be seen in the 0-minute photograph. (The spot on the sporangiophore in the 12-minute picture is a photographic imperfection.) The sporangium is above the top of the photograph. Between experiments 45 minutes was required for growth to return to normal. Each sporangiophore could be used for at most six experiments.

cuvette containing oil of the same refractive index ($n = 1.35$) as the cytoplasm to eliminate focusing by the sporangiophore and to minimize reflections. The monochromatic laser radiation was polarized in the x direction to further decrease reflections at the cell wall. Considerable attenuation was used to produce an average light intensity of 160 μw/cm^2 in the illuminated area (*10*). Several trials indicated that this amount was the lowest which consistently stimulated a tropic response larger than the random bending of 4° in 10 minutes. Blue light was also directed onto the fungus from opposite directions, which provided an adapting illumination of 0.16 μw/cm^2. Alignment, observation, and photography were performed in red light. Two telescopes and cameras were used to view and record the behavior of the sporangiophore in the xz and yz planes (*11*).

The sporangiophore was positioned in the x and z directions relative to the laser image as shown in Fig. 1A. All alignment was performed with the laser beam blocked. The angle between the laser image in the xz plane and the sporangiophore axis was adjusted to less than 2°. Location of the sporangiophore in the y direction was achieved by focusing the rear telescope on the beam waist and then moving the sporangiophore into focus. Consistent placement to within 100 μm of the beam waist was obtained.

Our results indicate that the microillumination technique is useful for probing the phototropic response. The hypothesis that phototropism results from greater growth in regions of more intense illumination had been suggested by several experiments. In those experiments the differential illumination was varied by altering the focusing by immersion in media of different refractive indices (*3*) or by ultraviolet light (*12*). However, the localization of the radiation was not specific, and the response was always in the xy plane. Thus, the present experiments provide a more rigorous confirmation of the hypothesis.

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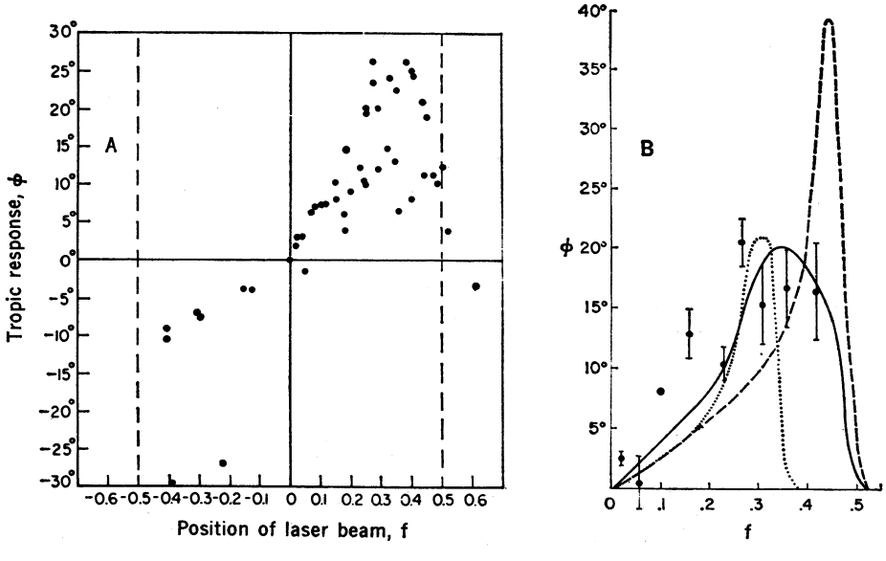

Fig. 3. (A) Tropic response in the xz plane as a function of the position of the beam. The parameter, f, is the distance of the laser beam from the sporangiophore axis divided by the sporangiophore diameter. (See Fig. 1B). Each point represents one trial. The vertical dashed line denotes the edge of the sporangiophore. (B) Average of data of 28 experiments. Only those with $f \leqslant 0.45$ and in which no lateral motion occurred during stimulation were included. Each point is an average of all data within intervals of 0.05 after correction for variation of sporangiophore diameters (*2, 4*). The experimental standard error of the points within each interval is indicated. Theoretical curves of tropic response are calculated for the following hypothetical distributions of pigment: (i) a 5-μm annulus near the inner (tonoplast) membrane, $0.30 \leqslant r \leqslant 0.35$, dashed curve; (ii) a 5-μm annulus near the outer wall, $0.45 \leqslant r \leqslant 0.50$, dotted curve; and (iii) throughout the cytoplasm, $0.30 \leqslant r \leqslant 0.50$, solid curve. The radius, r, is given in fractional units of the diameter. The curves were normalized by choosing a scale factor which gave the best least-squares fit with the experimental points.

References and Notes

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9. The temperature in the darkened room was between 19° and 21°C.
10. Partial saturation of the phototropic response would be expected if the entire sporangiophore were continuously illuminated with this intensity. A dose-response curve would provide further information regarding saturation effects with local irradiation.
11. To avoid retinal damage the laser beam was attenuated to less than 3×10^{-3} μw total power (the amount used in the experiment) before it was viewed directly with the telescope.
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