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INVITED REVIEW

PHOTOTROPISM OF PHYCOMYCES SPORANGIOPHORES

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Abstract—Sporangiophores (spphs) of *Phycomyces blakesleeanus* are positively phototropic to unilateral visible (blue) light over a range of fluence rates 10^{-9} to 1 W/m^2. The maximal bending angle is always 70-75° from the vertical. Many mutants with abnormal phototropism have been isolated. Complementation tests revealed that the genetic grouping is completely consistent with the phenotypic classification scheme, based on sensory responses other than those to light. The spph of the piloboloid mutant, the growth zone of which gradually ceases elongation but expands spherically, and the β-carotene-overproducing mutant show negative phototropism, in contrast to the wild type spph. We hypothesized that the phototropic orientation of spph is determined by the ratio of the maximal light fluence rate at the proximal side to that at the distal side of the spph. Based on this hypothesis, we found that the maximal bending angle was larger in thin spphs than in thick ones, and larger in spphs containing smaller amount of β-carotene than in carotene-rich spphs. In addition to our hypothesis, gravitropic experiments revealed that the maximal bending angle of the wild type spph results from a balance among positive phototropism, negative gravitropism, and the optical properties of the spph. For further advancement of this study, we developed a mutant with a high proportion of uninucleate spores, and designed an efficient microinjection method for obtaining transformants.

INTRODUCTION AND LIFE CYCLE

Much of the interest of our experimental material, the fungus *Phycomyces blakesleeanus*, stems from the concept of a "good model organism", that will be characterized by acceptance of varied experimental conditions and readiness for physiological and genetical analysis. About 35 years ago, Max Delbrück revaluated *Phycomyces* as a good model organism for behavioral and sensory physiology, just like phage and bacteria were so in genetics.

Phycomyces belongs to Zygomycetes and has two, asexual and sexual, life cycles (Fig. 1). Asexual life cycle is characterized by development of gigantic spphs which respond in various ways to several kinds of environmental stimuli. The most studied response has been phototropism. Sexual cycle is slow to complete, several months, but effective for meiotic analysis.

Asexual spphs emerged on nonseptated mycelia are unicellular and multinucleate cylinder with a diameter of about 100 μm, consisting of large central vacuole and peripheral cytoplasm. The development of the spph is divided into five stages based on its elongation and rotational behavior[1] (Fig. 1): longitudinal elongation at a pointed apex before sporangium formation and clockwise rotation when viewed from above (stage Ⅰ); radial expansion at the tip but no rotation during sporangium formation (stage Ⅱ); no elongation nor rotation after sporangium formation (stage Ⅲ); resumption of a slight elongation and counterclockwise rotation in a newly developed subapical growth zone beneath the sporangium (stage Ⅳa); subsequent active elongation and clockwise rotation (stage Ⅳb). The stage-Ⅳb spph elongates for several days at a steady growth rate (about 3 mm/h) and reaches more than 10 cm in length.

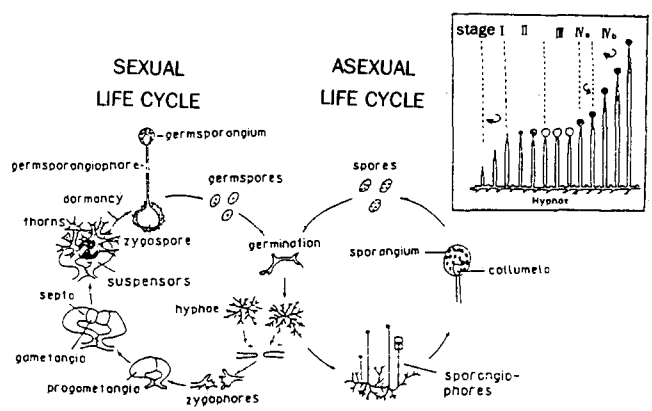

Figure 1. Asexual and sexual life cycles of *Phycomyces blakesleeanus* and diagram of developmental stages of spph (inset).

ANALYSIS OF PHOTOTROPISM OF WILD TYPE AND MUTANTS

The growth zone of spph, the pointed apex at stage I or the cylindrical subapical region at stage IVb, is nearly transparent and acts as a converging lens focusing the unilateral incident light at the distal side of the spph.[2] Thus, the distal side of spph receives a more intense light stimulus than the proximal side. The focusing advantage thus gained produces greater growth on the distal than the proximal side and is one of the principal mechanisms necessary for phototropism of this fungus.[3]

Max Delbrück and his coworkers collected many mutants with abnormal phototropism (genotype *mad*) by mutagenesis with NTG or ICR-170. The wild type spphs are positively phototropic in response to unilateral white light over a range of fluence rates (10^{-9} to 1 W/m^2), but all the *mad* mutants isolated are only insensitive to low fluence rate of light. These *mad* mutants were classified phenotypically into three groups on the basis of other responses to stimuli (Fig. 2).[4]

Class-1.1 mutants. normal gravitropism and chemotropism (avoidance), but no mycelial response to light, implying that these mutants are defective on the input side (photoreceptor system) of the "stimulus-transduction-response" chain.

Class-1.2 mutants. abnormal only in the phototropic response, implying that these mutants have some faults in the transdution system of light signal to the phototropic response.

Class-2 mutants. abnormal avoidance and gravitropism but nomal mycelial response to light, implying that these mutants are defective output side (response system) of the chain.

To gain some insights into the number of gene functions involved, it would be desirable to use heterokaryon complementation tests. In contrast to Ascomycetes and Basidiomycetes, Phycomycetes do not naturally anastomose to form heterokaryons, and we developed an efficient method which opened the way to numerous application.[5] We grafted two stage I spphs from different mutants and obtained heterokaryotic regenerates at the graft union (Fig. 3).

Thus, we tested the recovery of heterokaryons for phototropic response and found seven complementation groups, designated as *madA* to *madG*.[6-8] All *madA* to *madB* mutants coincided with the class 1.1 mutants, *madC* coincided with the class 1.2, and *madD* to *madG* mutants coincided with the class 2 (Fig. 2). Recombination analysis confirmed the existence of *madG* gene[9] and revealed that these seven complementation groups corresponded to seven unlinked genes.[9,10] Thus, a clear correspondence was found between the phenotypic classification and the complementation and recombination analysis. These clear-cut phenotypic and genotypic classifications are very useful for further analysis of these behavioral mutants.

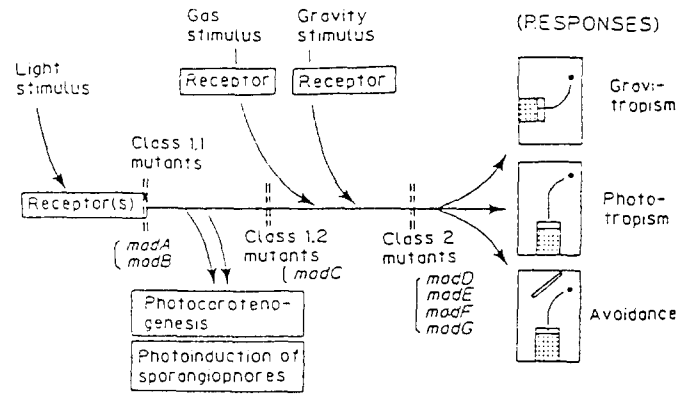

Figure 2. Network of stimulus-response system of *Phycomyces* spph. Modified from references 4 and 8.

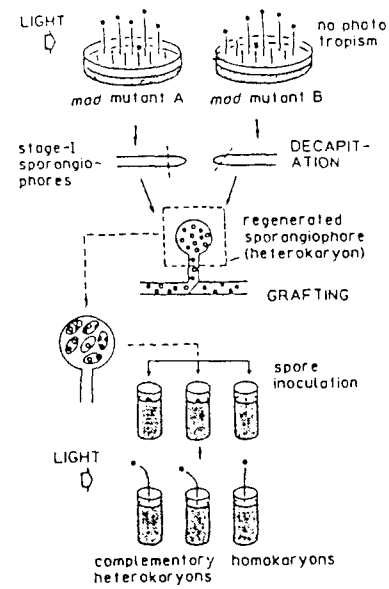

Figure 3. Procedure of heterokaryon formation and complementation test between two different mutants of *Phycomyces* with abnormal phototropism. Modified from references 5 and 7.

PHOTOTROPIC BEHAVIOR OF PILOBOLOID MUTANTS

We isolated many growth-zone defective mutants, piloboloid (genotype *pil*), by mutagenesis from the standard wild type and color mutants. These *pil* mutants are characterized by a gradual cessation of longitudinal elongation, and an increased rate of a

radial expansion in the growth zone, when the spphs have reached stage Ⅳb. We found that expression of the *pil* synchronizes greatly at low temperature (10°C)[11,12] and that the rotation of the *pil* spph at stage Ⅳb reverses its direction from clockwise to counterclockwise during the period of increased radial expansion.[13]

We found that the *pil* spph, in contrast to wild type spph, is always negatively phototropic to unilateral light when its diameter exceeds 210 μm.[14] Negative phototropism occurred regardless of their color and the *pil* gene they carried, and the reversal of rotation. Genetic complementation tests among *pil* mutants for negative phototropism and recombination analysis between *mad* mutant and *pil* mutant revealed that the negative phototropism of the *mad* mutant is governed by the phenotypic characteristics of *pil* (increase in diameter) but not by specific genes responsible for negative phototropism. We concluded that reverse phototropism of the *pil* mutant resulted from a loss of the convergent lens effect of the spph because of the increase in the spph diameter.

HYPOTHESIS FOR DETERMINATION OF PHOTOTROPIC DIRECTION AND MAXIMAL BENDING ANGLE

The discovery of negative phototropism in the *pil* mutant implied that the spph diameter or the length of the intracellular light path, is an important factor determining phototropic direction, positive or negative. We proposed a hypothesis that the direction of phototropism is determined by the ratio of the maximal light-fluence rates between the proximal side ($I_{P,max}$) and the distal side ($I_{D,max}$) of the spph (Fig. 4).[15,16]

When the wild type spph was unilaterally illuminated, the $I_{D,max}$ on the focal points of the distal side became much higher than $I_{P,max}$ on the central axis of the proximal side. We imagined that in the *pil* mutant the $I_{P,max}/I_{D,max}$ ratio must be reversed because of an increase in the intracellular light path, resulting in a loss of the focusing advantage on the distal side.

To estimate the $I_{P,max}/I_{D,max}$ ratio, we introduced some parameters[16], light attenuation coefficient (a), light divergence constant (k) mainly resulting from scattering, and the length of the intracellular light path (*2R;* spph diameter), in addition to refractive index of spph (1.38).[17] These parameters govern the magnitude of intracellular light attenuation, which greatly influences the $I_{P,max}/I_{D,max}$ ratio.

These parameters were theoretically[16] and experimentally[15] estimated. The $I_{P,max}$ was 0.9745 at the inner surface of the spph when light fluence rate 1.0 was used, because of the partial loss in light fluence rate caused by surface reflection. The effect of photoreceptors' orientation was ignored. We found theoretically that the light divergence constant can be estimated by measuring the distance of two focal points on the distal surface of the spph, and we experimentally estimated it to be about 0.058. We estimated the value of the light attenuation coefficient of the spph at stage Ⅳb by measuring transmission of the light beam, which was projected perpendicular to the developmental axis and passed through the center of the spph. It is about 6.42 mm^{-1}, when we simply assumed that the growth zone of the spph is an anatomically and optically homogeneous cylinder with a single refractive index.

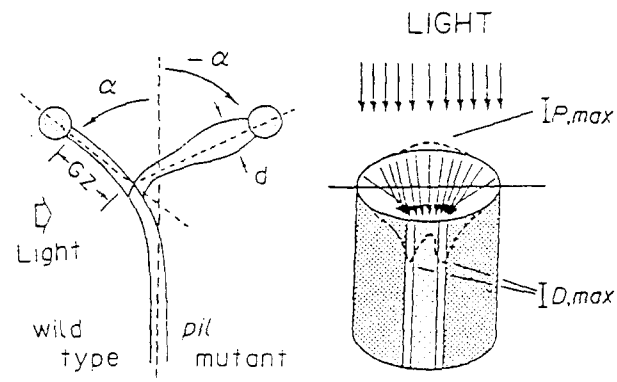

Figure 4. Schematic diagram of wild type and *pil* mutant spphs of *Phycomyces* showing positive and negative phototropism, respectively, and diagram of spph illuminated with unilateral light perpendicular to the developmental axis.

EVIDENCES SUPPORTING HYPOTHESIS

Using above parameters, we estimated the values of $I_{D,max}$ and the critical light attenuation (*2aR*), where $I_{P,max}$ and $I_{D,max}$ are equivalent.[15] The $I_{P,max}$ value is always constant, regardless of both the light attenuation coefficient and the spph diameter. The critical *2aR* value was theoretically about 1.39. If our hypothesis is valid, spphs with a *2aR* value below 1.39 receive the most intense light on the distal side ($I_{P,max} < I_{D,max}$) when illuminated unilaterally and their phototropism is positive. Spphs with a value above 1.39, however, receive the most intense light on the proximal side ($I_{P,max} > I_{D,max}$) and phototropism is negative. We applied this *2aR* value to the *pil* spphs and found that almost all *pil* spphs with diameter larger than 210 μm had a *2aR* value above 1.39. We already know that these *pil* spphs were negatively phototropic, supporting our hypothesis.

A *2aR* value larger than 1.39 will be obtained by an increase in a light attenuation-coefficient value (*a*), instead of an increase in *2R* (*pil* mutant). We

found that the β-carotene overproducing mutant (*carS*) with a normal diameter had a *2aR* value above 1.39, because of an increase in light attenuation coefficient (*a*) resulting from the accumulation of excess β-carotene and that these spphs were negatively phototropic.[15]

APPLICATION OF HYPOTHESIS TO BENDING SPORANGIOPHORE

In the spph bending toward the light source, a change of the incident angle of light to the spph axis, concomitant with a change of bending angle of spph, causes a change of light-path length and a change of light-distribution profile on both the proximal and distal sides of the the spph, influencing the $I_{P,max}/I_{D,max}$ ratio. The spph continues the positive phototropism as long as $I_{D,max}$ is larger than $I_{P,max}$ and stops bending when these values become equivalent, where is the point of the maximal bending angle of the spph.

A theoretical analysis revealed that the $I_{P,max}$ is equivalent to $I_{D,max}$ at the bending angle of 72° for the wild type spph with a fixed light divergence constant (0.058), light attenuation coefficient (6.42 mm^{-1}), and diameter (100 μm).[18] This result implies that the spph was unable to bend beyond the critical bending angle (72°), because of the optical properties of the spph.

We also found theoretically that the maximal bending angle was larger in thin spphs (a small intracellular light path) than in thick ones (a large intracellular light path), and larger in the spphs containing a small amount of β-carotene (a small light-attenuation coefficient) than in those containing a large amount of it (a large attenuation coefficient). We experimentally confirmed them.[18]

The involvement of optical properties of spph in the determination of phototropic bending angle was supported by the fact that the maximal bending angle of the spph illuminated unilaterally from a fixed side on a rotating clinostat was about 72°.[19] Our hypothesis was also supported when we applied it to the negatively-bending spphs of *pil* mutant with a diameter exceeding 210 μm.[20] Beyond 210 μm, the bending angle approached asymptotically to -40° as the diameter approached 360 μm. Beyond 360 μm, the bending angle decreased rapidly to about -90°. This diphasic pattern of the negative phototropism was experimentally confirmed, agreeing with the theoretical prediction.

EFFECT OF NEGATIVE GRAVITROPISM ON PHOTOTROPISM

To investigate the influence of negative gravitropism on the determination of the maximal phototropic bending angle, we placed the spphs vertically or horizontally and illuminated them from different directions. We found different maximal bending angles among the spphs. These results implied that the maximal bending angle of the spph resulted from a balance among a positive phototropism, a negative gravitropism, and the optical properties of the spph, probably the $I_{P,max}/I_{D,max}$ ratio.[19]

FOR FURTHER STUDY OF PHOTOTROPIC RESPONSE IN PHYCOMYCES:

For study of photosensory physiology, mutants are quite useful and strategical tools. The mutants isolated are numerous but still insufficient, because we have not isolated any completely insensitive mutants to light or gravity. For isolating such mutants at high efficiency, haploid and uninucleate spores are more convenient because most of the mutants are recessive. *Phycomyces* spores are haploid but almost all spores are multinucleate. We isolated a mutant which produced uninucleate spores with a high proportion of about 50%.[21]

In *Phycomyces*, the analysis of the sensory system at molecular level is quite limited. Cloning the genes involved in phototropism and introducing the cloned genes into *Phycomyces* by transformation are quite powerful tools for identifying their functional roles. We developed a high efficient method to obtain transformants by microinjecting exogenous genes into young sporangia isolated from stage II-III spphs (Fig. 1).[22] Most of the sporangia treated developed normally the spores and some of the spores expressed the trait of the injected genes. The present transformation efficiency was about several hundred times higher than that obtained with the protoplast method.[23] This method is likely to open the way for further analysis of phototropic response of this fungus at molecular level.

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