

Wavelength Dependence of Dark Adaptation in *Phycomyces* Phototropism

PAUL GALLAND, ABHIJIT S. PANDYA, and
EDWARD D. LIPSON

From the Department of Physics, Syracuse University, Syracuse, New York 13210

ABSTRACT The wavelength dependence of phototropic dark adaptation in *Phycomyces* was studied between 347 and 545 nm. Dark adaptation kinetics were measured for wavelengths of 383, 409, 477, and 507 nm in the intensity range from 6.2×10^{-2} to 2×10^{-7} $\text{W} \cdot \text{m}^{-2}$. At these wavelengths, dark adaptation follows a biexponential decay as found previously with broadband blue light (Russo, V. E. A., and P. Galland, 1980, *Struct. Bonding.*, 41:71; Lipson, E. D., and S. M. Block, 1983, *J. Gen. Physiol.*, 81:845). We have found that the time constants of the fast and slow components depend critically on the wavelength. At 507 nm, dark adaptation kinetics were found to be monophasic. The phototropic latency after a step down by a factor of 500 was measured for 19 different wavelengths. Maximal latencies were found at 383, 477, and 530 nm; minimal latencies were found at 409 and 507 nm. With irradiation programs that employ different wavelengths before and after the step down, the dark adaptation kinetics depend critically on the sequence in which the two wavelengths are given. We have found too that not only do the adaptation kinetics vary with wavelength, but so also do the phototropic bending rate and the phototropic latencies in experiments without intensity change. The results imply that more than one photoreceptor is mediating phototropism in *Phycomyces* and that sensory adaptation is regulated by these photoreceptors.

INTRODUCTION

The sporangiophore of the fungus *Phycomyces* senses blue light over an enormous intensity range from 10^{-9} to 10 $\text{W} \cdot \text{m}^{-2}$ (Bergman et al., 1969). Within this range, changes of the light intensity can cause a transient light growth response under symmetrical illumination or phototropism when unilateral light stimuli are used. Over a wide intensity range, the response to light depends on the subjective stimulus, defined as the ratio between the stimulus intensity and the constant intensity to which the specimen was exposed previously (Delbrück and Reichardt, 1956; Foster and Lipson, 1973). In order for the sporangiophore to compute the ratio of two intensities, the light intensity to which the system had been exposed previously has to be represented and stored inside the cell; this is the main function of the adaptation mechanism.

Address reprint requests to Dr. Edward D. Lipson, Dept. of Physics, Syracuse University, Syracuse, NY 13210.

Under steady state conditions, the "level of adaptation" is defined formally as the light intensity with which the system has come into equilibrium. In nonequilibrium conditions, one can infer the virtual level of adaptation by measuring the reaction to a series of standardized test pulses (Delbrück and Reichardt, 1956; Lipson and Block, 1983). The molecular basis of adaptation is unknown, even though the time courses of dark and light adaptation have been measured in some detail (Delbrück and Reichardt, 1956; Russo and Galland, 1980; Lipson and Block, 1983; Galland and Russo, 1984).

Genetic evidence shows that adaptation is mediated at least partially at the level of the photoreceptor: behavioral mutants with altered action spectra also have altered adaptation kinetics (Russo and Galland, 1980; Galland, 1983; Galland and Russo, 1984; Lipson et al., 1984). However, there has been no direct physiological evidence that the photoreceptor system is involved in sensory adaptation. We present such evidence here in a study of the wavelength dependence of phototropic dark adaptation. The results indicate that multiple photoreceptors must operate in *Phycomyces* phototropism and that sensory adaptation is regulated by the interaction of these receptors.

MATERIALS AND METHODS

Strain

The wild-type strain NRRL1555 (-), originally obtained from the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, IL, was used in this work. Media and growth conditions were as described by Lipson and Terasaka (1981). Sporangiohores were grown under fluorescent white light (Cool White F15/T8/CW; Sylvania/GTE, Exeter, NH). The effective broadband blue light intensity was $0.2 \text{ W} \cdot \text{m}^{-2}$.

Phototropism Chamber

The experiments were performed in a cylindrical aluminum chamber with inside diameter 5.1 cm and height 6.3 cm. The outside diameter was 10 cm and the outside height 12 cm. The chamber had four circular windows (diameter 4.5 cm) on the sides and a removable one on the top. The temperature inside the chamber was kept at $21.0 \pm 0.1^\circ\text{C}$ with a refrigerated circulator.

For each experiment, a shell vial containing a single sporangiophore was inserted from above. The vial was supported by a holder that could be adjusted vertically by a micrometer and which could also be rotated around a vertical axis by a small motor (10 rpm). In addition, the base plate of the chamber that held the vial holder and the motor could be displaced to adjust the lateral position of the sporangiophore. All these adjustments of the sporangiophore position could be made without opening the chamber. No leakage of light or air occurred during these movements or at any other time. Three windows of the chamber were made of red acrylic plastic (S10218 Mitsubishi Rayon Co., Japan, obtained from Payne Glass Co., Pasadena, CA); the plastic has a sharp cutoff at 610 nm.

The actinic light was given through the fourth window, which was made of transparent acrylic plastic. In front of this window, a filter holder was attached to the chamber; the holder accommodated heat-absorbing filters, gray filters, and a slider in which the interference filters were placed individually. With the slider, the wavelength of the actinic light could be changed quickly. The interference filters (Balzers, obtained from Rolyn Optics, Covina, CA) had a bandwidth of 9–12 nm. Two heat-absorbing filters (obtained

from Rolyn Optics, catalog number 65-3035) were inserted into the light path; they had 0.5% transmission for wavelengths above 1,000 nm.

Sporangiophores were adapted for 45 min while they were rotating. The phototropic stimulus was given simply by stopping the rotation. Phototropism was observed and recorded with a standard video camera (RCA TC2011; RCA Electro-Optics & Devices, Lancaster, PA), a time-lapse video recorder (RCA TC3100A), and two video monitors. One monitor (RCA TC1109) was used for direct viewing during the experiment; the other (RCA TC1212) was used for playback. The video system is described in detail elsewhere (Lipson and Häder, 1984). During the playback of each experiment, a ruler attached to a rotary potentiometer was maintained parallel to the upper part of the bending sporangiophore. The orientation of the ruler was thereby converted into a voltage and sent to a strip-chart recorder.

The light source was a 500-W slide projector (500 RR-2; Standard). Light intensities were adjusted with a variable transformer and calibrated neutral-density filters. Light intensities were measured with a photodiode (UV-100; United Detector Technology, Santa Monica, CA) and an electrometer (610B; Keithley Instruments, Inc., Cleveland, OH). The photodiode was calibrated as a function of wavelength with an Eppley thermopile (Eppley Laboratory, Inc., Newport, RI).

Data Analysis

The dark adaptation data were fit by nonlinear least-squares analysis (Hamilton, 1964) to the following biexponential equation:

$$A = A_1 \exp[-(t - t_0)/b_1] + A_2 \exp[-(t - t_0)/b_2].$$

The independent variable was the adaptation level, A ; the dependent variable was the phototropic delay, t . The implicit equation above was solved numerically by Newton's method. In the nonlinear fitting procedure, we needed the derivatives of the implicit function $t(A)$ with respect to the parameters; these derivatives were also obtained by numerical methods. The computations were performed in the language APL on an IBM 4341 computer. The fitting procedure included statistical weighting according to the standard errors for the individual data values for the phototropic delay. The program provided estimates of the parameters A_1 , A_2 , b_1 , b_2 , and t_0 as well as the standard errors (and covariances) of these estimates.

RESULTS

The phototropic latency method provides a convenient way to follow the time course of dark adaptation (Bergman et al., 1969; Russo and Galland, 1980): sporangiophores are first adapted symmetrically to a certain light intensity I_0 and then they are irradiated unilaterally at intensity I . The phototropic latency is defined as the time that elapses from the beginning of the unilateral stimulus to the beginning of the steady state bending, determined conventionally by extrapolation as shown in Fig. 1. The phototropic latency is a function of the ratio of I to I_0 (Bergman et al., 1969; Russo and Galland, 1980). After the step down, phototropic bending commences when the internal state of adaptation becomes equilibrated with the new intensity, I ; in this situation, the level of adaptation is equal to the intensity level (Delbrück and Reichardt, 1956). Because of this relationship, the experimental variable, I , can be replaced by A .

Previous adaptation studies had been done with broadband blue light (Delbrück and Reichardt, 1956; Russo and Galland, 1980; Galland and Russo, 1984)

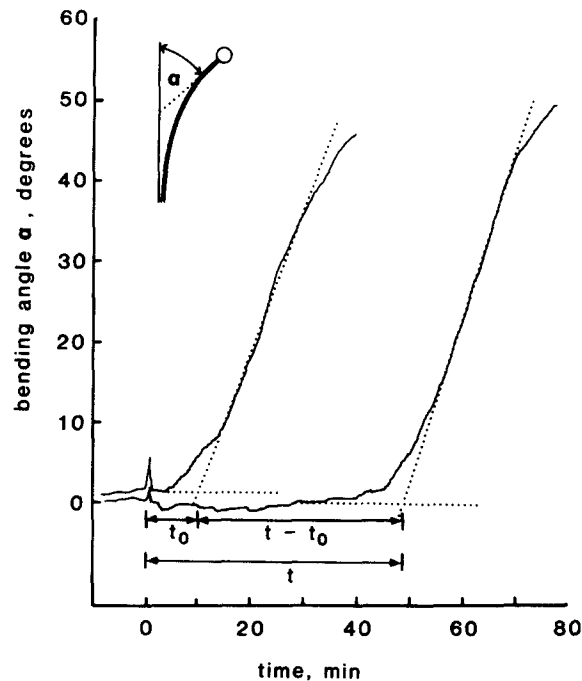


FIGURE 1. Determination of the phototropic latency. Each sporangiophore was adapted symmetrically to an intensity of $1.3 \times 10^{-2} \text{ W} \cdot \text{m}^{-2}$ (452 nm) for 45 min and was then exposed to unilateral light of the same intensity (left curve) or else 500-fold-reduced intensity (right curve). The phototropic latency is determined by extrapolation to the baseline as shown. The quantities t , t_0 , and $(t - t_0)$ are obtained from Eq. 1: t is the phototropic latency in experiments involving a step down of the intensity of the unilateral light; t_0 is the intrinsic phototropic latency in experiments without intensity range; $(t - t_0)$ is the adjusted latency.

or with 488-nm light (Lipson and Block, 1983). Here we have tested the phototropic latency as a function of the wavelength.

Fig. 2 shows the kinetics of phototropic dark adaptation at 383, 409, 477, and 507 nm over an intensity range of 10^5 . At these wavelengths, sporangiophores were adapted to the equivalent subjective intensity, according to the photogeotropic action spectrum (Galland, 1983). The time course of dark adaptation depends strongly on the wavelength. For 383, 409, and 477 nm, we obtained biphasic kinetics, as found before with broadband blue light (Russo and Galland, 1980). The kinetics of the adaptation level, A , were fit to the empirical formula

$$A = A_1 \exp[-(t - t_0)/b_1] + A_2 \exp[-(t - t_0)/b_2]. \quad (1)$$

The equation holds only for $t \geq t_0$. The constant t_0 is the phototropic latency obtained in experiments without intensity change (see Fig. 1). In this equation, the adaptation level A is formally expressed as the sum of two distinct adaptation levels, A_1 and A_2 . Table I shows that the dark adaptation constants b_1 and b_2 , the parameter t_0 , and the expression $A_1/(A_1 + A_2)$ all depend on the wavelength.

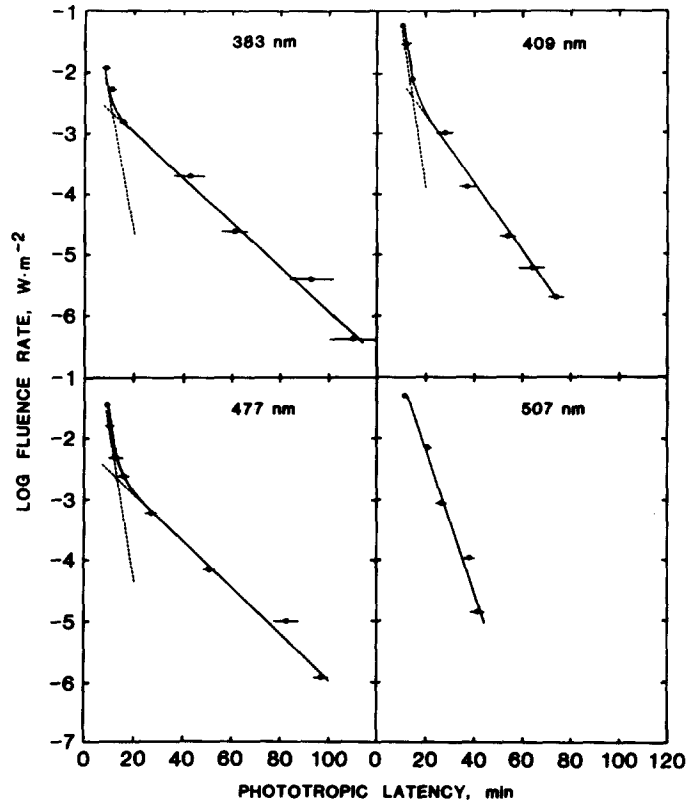


FIGURE 2. Phototropic dark adaptation kinetics at four wavelengths. Sporangiophores were adapted symmetrically to the intensities corresponding to the highest points of the curves. After 45 min of adaptation, unilateral light of the intensities indicated on the ordinate was given and the phototropic latency was determined. Each horizontal bar represents the standard error of at least four experiments.

Fig. 3 shows the intrinsic phototropic latency t_0 as a function of wavelength. In these experiments, sporangiophores were adapted to equivalent subjective intensities according to the photogeotropic action spectrum (Galland, 1983). We found unexpectedly that even the intrinsic phototropic latency depends strongly on the wavelength; it is maximal at 414 nm, shows secondary maxima at 365, 394, 452, and 507 nm, and decreases in the near ultraviolet and in the green part of the spectrum. Fig. 4 shows the wavelength dependence of the bending rate. Because the curves for the bending rate differ from the curve for the intrinsic phototropic latency (Fig. 3), the two variables are probably under the control of different processes.

We measured the phototropic latencies as a function of wavelength for constant steps down by a factor of 500. As above, the sporangiophores were adapted to the equivalent subjective intensity. In order to obtain the actual adaptation time, we subtracted the intrinsic latency, t_0 , shown in Fig. 3, from the observed phototropic latency, t . We call $(t - t_0)$ the "adjusted latency"; it is the time the

TABLE I
Wavelength Dependence of Adaptation Parameters in Eq. 1

Wavelength	b_1	b_2	t_0	$A_1/(A_1 + A_2)$
nm	min	min	min	
383	1.9 ± 2.0	11.7 ± 1.4	8.4 ± 1.0	0.8 ± 0.12
409	1.4 ± 0.6	7.8 ± 0.8	11.2 ± 0.8	0.9 ± 0.05
477	1.7 ± 0.7	11.4 ± 0.5	8.9 ± 0.9	0.91 ± 0.02
507	3.7 ± 0.3	—	12.2 ± 0.9	1.00
383→409	0.6 ± 0.8	11.0 ± 2.9	16.3 ± 1.2	0.97 ± 0.01
409→383	6.2 ± 0.3	—	11.1 ± 0.7	1.00

Dark adaptation kinetics with only one time constant are listed under b_1 . In these cases, $A_1/(A_1 + A_2) = 1$ by definition. Here the values of t_0 are estimates obtained by the fitting procedure (see Materials and Methods) and therefore differ slightly from the measured values shown in Fig. 3. Standard errors were obtained from the least-squares analysis described in Materials and Methods. In the last two experiments employing two wavelengths, the first one indicates the wavelength of the preadaptation, and the second one the wavelength of the unilateral light.

sporangiophore needs to adapt to the new intensity level after the step down. The adjusted latency depends greatly on the wavelength; it shows maxima at 386, 477, and 530 nm and minima at 409 and 507 nm (Fig. 5).

To test if the wavelength dependence of the phototropic latency varies with the intensity range, we measured the phototropic latency for 383 and 409 nm at different intensities. Table II shows the phototropic latencies to steps down by a factor 500 at three different intensities. The different latencies at 383 and 409 nm are essentially independent of the intensity range. This result shows that the

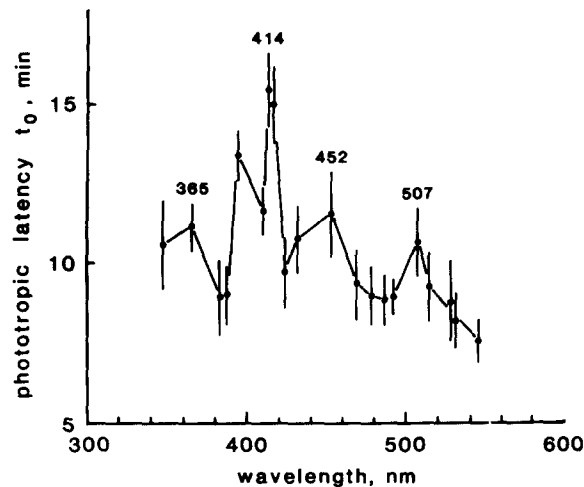


FIGURE 3. Wavelength dependence of the intrinsic phototropic latency, t_0 , for experiments in which the intensities of the preadapting light and the unilateral light were identical. Sporangiophores were adapted symmetrically for 45 min and were then exposed unilaterally to the same wavelength and intensity. At all wavelengths, the subjective intensity was the same (equivalent to $1.3 \times 10^{-2} \text{ W} \cdot \text{m}^{-2}$ at 450 nm; the other intensities were scaled according to the photogeotropic action spectrum).

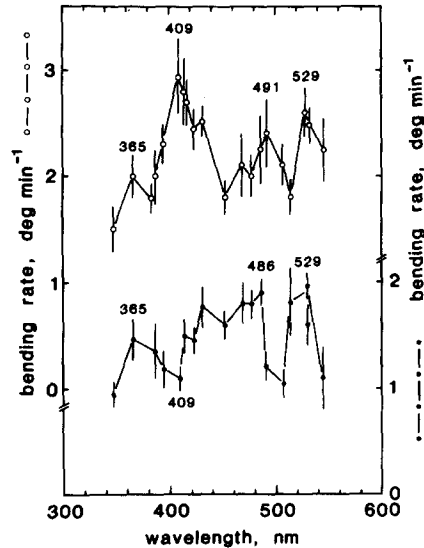


FIGURE 4. Wavelength dependence of phototropic bending rate. Open symbols: from experiments shown in Fig. 3; intensities of the preadaptation light and unilateral light were identical; standard error of at least eight experiments. Closed symbols: bending rate after a step down of intensity by a factor of 500; from experiments shown in Fig. 5. Standard errors are shown for at least six experiments.

wavelength dependence of the phototropic latency is not due simply to errors in the adjustment of the subjective intensity.

We performed experiments with two wavelengths to determine whether the dark adaptation parameters b_1 , b_2 , t_0 , and $A_1/(A_1 + A_2)$ depend on the wavelengths given before or after the step down. Fig. 6 shows the dark adaptation kinetics for sporangiophores that were either adapted symmetrically at 409 nm and then exposed to unilateral light of 383 nm (Fig. 6*a*) or adapted symmetrically at 383 nm and then exposed to unilateral light of 409 nm (Fig. 6*b*). In these experiments, the sporangiophores were adapted to equivalent subjective intensities, according to the photogeotropic equilibrium action spectra (Galland, 1983). The kinetics in Fig. 6, *a* and *b*, differ from the kinetics with monochromatic irradiation (Fig. 2) and differ from each other. This shows that the dark adaptation kinetics are influenced both by the wavelength during preadaptation and by the wavelength after the step down. Sporangiophores that were adapted at 409 nm and then irradiated with 383 nm show a monophasic dark adaptation with a time constant of $b = 6.2$ min; sporangiophores treated in the reciprocal experiment (Fig. 6*b*) show a biphasic decay with $b_1 = 0.6$ min and $b_2 = 10.9$ min (Table I). The latter kinetics have two further remarkable features: (*a*) the proportion $A_1/(A_1 + A_2)$ is higher than in experiments with monochromatic light (Table I), and (*b*) $t_0 = 16.3$ min, which is 4.7 min longer than the phototropic latency obtained in the monochromatic experiments (Table I).

To ensure that this abnormally long latency of 16.3 min was not due to an error in the subjective intensity, we studied the kinetics further by including

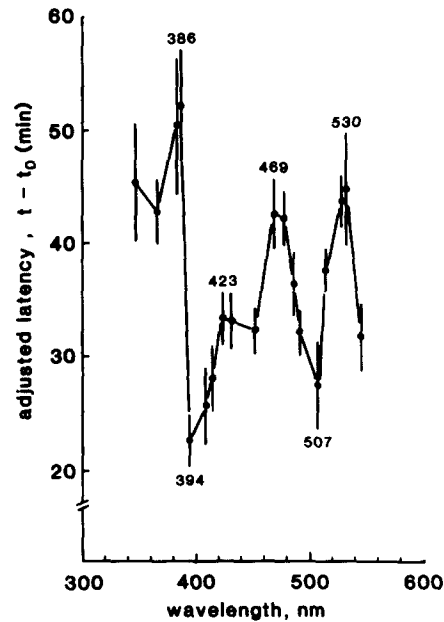


FIGURE 5. Wavelength dependence of the adjusted latency ($t - t_0$). Sporangiophores were adapted bilaterally for 45 min and were then exposed to unilateral light of 500-fold-reduced intensity; the wavelength was not changed during each experiment. At all wavelengths, the subjective intensity was the same; the intensity of the adapting light at 450 nm was $1.3 \times 10^{-2} \text{ W} \cdot \text{m}^{-2}$. Error bars represent standard errors of at least six experiments.

steps up (Fig. 6, *a* and *b*). For steps up in intensity, the phototropic latencies increase in accordance with previous results obtained with broadband blue light (Russo and Galland, 1980; Galland and Russo, 1984). The intersection of the light adaptation and the dark adaptation kinetics shows a minimum in Fig. 6*a* (shift from 409 to 383 nm); the minimum latency is between 9 (experimental) and 11 min (by extrapolation), which is similar to the latencies in experiments with monochromatic light of 409 or 383 nm and no intensity change (Figs. 2

TABLE II
Intensity Dependence of the Phototropic Latency

Wavelength	Preadaptation intensity	Phototropic latency
<i>nm</i>	$\text{W} \cdot \text{m}^{-2}$	<i>min</i>
383	1.6×10^{-5}	65.2 ± 4.6
383	1.2×10^{-2}	61.3 ± 4.8
383	1.2	65.0 ± 3.5
409	8.7×10^{-5}	41.4 ± 2.1
409	6.2×10^{-2}	37.5 ± 3.4
409	4.3	45.6 ± 3.5

Sporangiophores were adapted symmetrically to the indicated intensities and were then exposed unilaterally to light of 500-fold-reduced intensity. The standard errors of at least four experiments are shown.

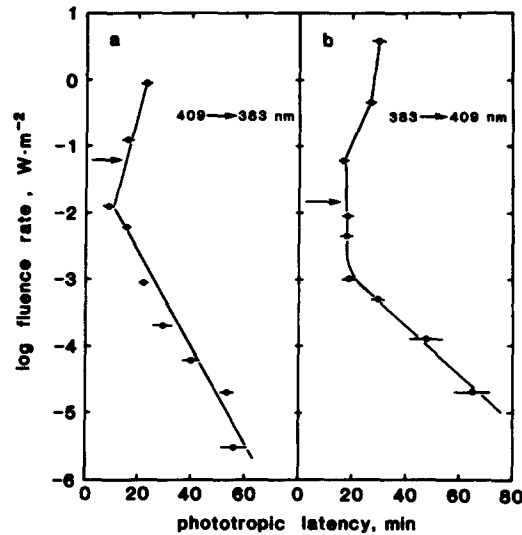


FIGURE 6. Kinetics of phototropic dark adaptation involving two wavelengths. (a) Sporangiohores were adjusted to $6.2 \times 10^{-2} \text{ W} \cdot \text{m}^{-2}$ at 409 nm; after 45 min of adaptation, unilateral light of 383 nm was given at the indicated intensities. (b) Sporangiohores were adapted to $1.2 \times 10^{-2} \text{ W} \cdot \text{m}^{-2}$ at 383 nm; after 45 min of adaptation, unilateral light of 409 nm was given at the indicated intensities. The intensities chosen for the adaptation at the two wavelengths are equivalent intensities according to the photogeotropic action spectrum. Arrows indicate the respective intensities of the preadapting wavelength. Bars represent the standard error of at least four experiments.

and 3). This result shows that under these conditions the sporangiophore recognizes 409 and 383 nm as having the same subjective intensities. However, in the reciprocal experiment (Fig. 6b), the effects of 383 and 409 nm are not equivalent, because the minimum latency was 16.3 min.

DISCUSSION

In the past, sensory adaptation of *Phycomyces* was studied in experiments employing either broadband blue light (Delbrück and Reichardt, 1956; Russo and Galland, 1980; Galland and Russo, 1984) or monochromatic (488 nm) blue light (Lipson and Block, 1983). This had seemed sufficient then, since the available evidence had suggested only one blue light photoreceptor in *Phycomyces*, most likely a flavoprotein (Otto et al., 1981). Under this assumption, no wavelength dependence of adaptation would be expected, whether the adaptation mechanism were at the receptor site itself or at a later step behind the photoreceptor.

The discovery that dark adaptation is wavelength dependent allows us to rule out several specific models proposed for adaptation. In one model, the level of adaptation was linked to the regeneration process of the bleached photoreceptor (Lipson, 1975); in two other models, the adaptation was attributed to processes in the transduction chain downstream from the receptor (Delbrück and Reichardt, 1956; Russo, 1980). In all three models, the adaptation mechanism would

register a change in the number of excited receptor molecules after a light intensity change. If only one pigment were mediating phototropism, then the relative change in the number of excited receptors would be wavelength independent. However, the data shown in this work indicate clearly that the dark adaptation process depends on the wavelength. Therefore, we conclude that the wavelength dependence of adaptation is attributable to the interaction of different pigments.

The data in Figs. 3–5 do not represent true action spectra, since the wavelength dependence was obtained without fluence response curves and the ordinates in these curves do not represent relative quantum efficiencies.

The fact that both t_0 (Fig. 3; intrinsic phototropic latency observed in experiments without intensity change) and the bending rate (Fig. 4) depend on wavelength corroborates the conclusion that more than one photoreceptor mediates phototropism in *Phycomyces* (Galland, 1983; Lipson et al., 1984). It is noteworthy that the prominent 530-nm peak found for the adjusted latency (Fig. 5) is also present for the wavelength dependence of the bending rate. We checked for possible correlations between the bending rates and the adjusted latencies, but could not find a clear relationship. The 530-nm peaks in Figs. 4 and 5 are absent in the action spectra of (a) phototropic balance (Lipson et al., 1984), (b) photogeotropic equilibrium (Galland, 1983; Lipson et al., 1984), and (c) light growth response (Delbrück et al., 1976). The prominent 414-nm peak found for the intrinsic latency (Fig. 3) is present also in the photogeotropic equilibrium action spectrum (Galland, 1983; Lipson et al., 1984). We do not know why the wavelength dependencies of the intrinsic latency, bending rate, and adaptation time all are different.

The wavelength dependence of t_0 and the bending rate could be caused by screening pigments as well as by the wavelength dependence of the refractive index of the cytoplasm. These parameters could cause changes in the relative intensity and the width of the focused band. (The sporangiophore of *Phycomyces* has the optical properties of a cylindrical lens; the actinic light is focused on the distal side into a narrow bright band of about six times higher light intensity than the proximal side [Dennison and Foster, 1977].) Zankel et al. (1967) and Jesaitis (1974) measured absorption spectra of the growing zones of *Phycomyces* sporangiophores that were grown on the same type of medium we used. They found an absorption spectrum that was highest at 400 nm (absorbance 0.16) and decreased gradually out to 560 nm (absorbance 0.06). Therefore, the sharp peaks found in Figs. 3 and 4 cannot be explained by preferential screening of substances in the growing zone. This conclusion is corroborated by another argument: the intrinsic latency and bending rate were measured at equivalent subjective intensities as determined from the photogeotropic equilibrium action spectrum (Galland, 1983). Since this action spectrum is subject to the same artifacts of screening and wavelength-dependent refractive index, the adjustment of equivalent intensities in our experiment should eliminate these disturbing factors. Therefore, the wavelength dependence we found cannot be explained on the basis of these artifacts but must be rather attributed to some state of the photoreceptor system that differs from the sensory adaptation system.

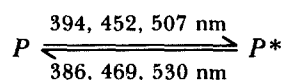
Orientation of dichroic photoreceptors could cause wavelength dependence of t_0 and the bending rate. The results of Jesaitis (1974) and Hertel (1980) suggest that the photoreceptor molecules are indeed oriented. Their data analysis was, however, based on the assumption of a single photoreceptor in *Phycomyces*. It is not clear what their data mean with respect to the relative orientation of the multiple photoreceptors to each other, and we are therefore unable to predict the influence of dichroism on t_0 and the bending rate. Could the wavelength dependence of the adaptation kinetics be explained by dichroic photoreceptors assuming only one type of receptor molecule? This seems very unlikely because (a) receptor orientation might play a role in regulating the intrinsic latency and/or the bending rate for which spatial differences between the proximal and distal sides must be detected, but it could not play a role when relative intensity differences must be registered as a function of time, and (b) such an hypothesis could not explain the results with dichromatic irradiation (Fig. 6), which demonstrate that the temporal sequence in which the two wavelengths are given is crucial. The latter results are the best evidence that more than one photoreceptor is involved in dark adaptation; if only one photoreceptor were mediating both phototropism and adaptation, then the phototropic latencies should have been identical in the reciprocal experiments shown in Fig. 6.

The fact that the adjusted phototropic latency, $(t - t_0)$, the adaptation constants b_1 and b_2 , and also $A_1/(A_1 + A_2)$ can be influenced by the wavelength given after the step down of light intensity has several interesting implications. These findings mean that after a step down of intensity the sporangiophore can sense the wavelength of the actinic light even though no phototropism occurs. In other words, the absence of a response during the latency time does not imply a lack of light perception. This indicates an input mechanism that is independent of the established level of adaptation and that can be recognized only through its effect on the parameters b_1 , b_2 , t , and $A_1/(A_1 + A_2)$. This element in the transduction chain of *Phycomyces* that controls adaptation must be associated with the photoreceptor system since it shows wavelength dependence. We conclude therefore that the photoreceptors are sensitive during the period of phototropic latency when no reaction occurs. This means that pigment regeneration cannot be the rate-limiting step in the adaptation process. Since pigment regeneration is a dark process, no wavelength dependence of the phototropic delay would be expected.

Recently, independent evidence for the presence of multiple photoreceptors in *Phycomyces* was found through the analysis of phototropic action spectra (Galland, 1983; Lipson et al., 1984). It was proposed that *Phycomyces* has either two (or more) antagonistic receptors (multireceptor hypothesis) or else one receptor with photochromic properties (photochrome hypothesis). Photochromic blue light receptor pigments were first proposed by Hartmann (1977) and suggested again by other authors (Löser and Schäfer, 1980; Hertel, 1980). In both hypotheses, adaptation could occur at the level of the photoreceptors. Under the multireceptor hypothesis, one can assume that the antagonistically acting photoreceptor (which controls synthesis of an inhibitor, for instance) represents the adaptation. Formally, this is very similar to previous adaptation

models (Delbrück and Reichardt, 1956; Russo, 1980) in which the output of the transduction chain is controlled by the ratio of a promoter p and an inhibitor i (adaptation); both molecules in these models were thought to be under the control of a single photoreceptor. In the multireceptor hypothesis, the promoter and inhibitor would be controlled by different photoreceptors. In the photochrome hypothesis, one can assume that the percentage of the biologically active form of the photochrome determines the dark adaptation constants.

In this photochrome hypothesis, Hartmann (1977) postulated that the biologically inactive form of the pigment, P , has an absorption maximum at 450 nm and two shoulders at 410 and 480 nm, while the biologically active form, P^* , should have absorption maxima at 380 and 520 nm. Thus, these peaks of the hypothetical photochrome are close to the wavelengths at which the adjusted latency is maximal (Fig. 5). The long phototropic delay obtained at 469 nm would then indicate that the biologically active form also absorbs at this wavelength. One can hypothesize that the slow dark adaptation kinetics are caused by a shift of the photoequilibrium from the P form to the P^* form:



This model would imply either that the P form slows down dark adaptation or that the P^* form accelerates dark adaptation. This conclusion is supported qualitatively by the results of the two-wavelength experiments (Fig. 6): a shift from 383 nm during preadaptation to 409 nm results in very fast dark adaptation ($b_1 = 0.6$ min), while in the reciprocal experiment a longer time constant ($b = 6.2$ min) is obtained. However, in this qualitative outline of events, the wavelength dependence of the other parameters, t_0 , b_2 , and $A_1/(A_1 + A_2)$, is not included.

The data presented here are the first physiological evidence that sensory adaptation in *Phycomyces* is controlled by the photoreceptor system. The results are incompatible with the classical one-photoreceptor model and lend strong support for the existence of either a multireceptor system or a photoreceptor with photochromic properties, or even a system which includes both these features.

This project was supported by National Institutes of Health grant GM29707 and an Alfred P. Sloan Foundation Fellowship to E. D. L.

Original version received 7 April 1984 and accepted version received 4 June 1984.

REFERENCES

- Bergman, K., P. V. Burke, E. Cerdá-Olmedo, C. N. David, M. Delbrück, K. W. Foster, E. W. Goodell, M. Heisenberg, G. Meissner, M. Zalokar, D. S. Dennison, and W. Shropshire, Jr. 1969. *Phycomyces*. *Bacteriol. Rev.* 33:99–157.
- Delbrück, M., A. Katzir, and D. Presti. 1976. Responses of *Phycomyces* indicating optical excitation of the lowest triplet state of riboflavin. *Proc. Natl. Acad. Sci. USA.* 73:1969–1973.
- Delbrück, M., and W. Reichardt. 1956. System analysis for the light growth reactions of

- Phycomyces*. In Cellular Mechanisms in Differentiation and Growth. D. Rudnick, editor. Princeton University Press, Princeton, NJ. 3–44.
- Dennison, D. S., and K. W. Foster. 1977. Intracellular rotation and phototropic response of *Phycomyces*. *Biophys. J.* 18:103–123.
- Foster, K. W., and E. D. Lipson. 1973. The light growth response of *Phycomyces*. *J. Gen. Physiol.* 66:590–612.
- Galland, P. 1983. Action spectra of photogeotropic equilibrium in *Phycomyces* wild type and three behavioral mutants. *Photochem. Photobiol.* 37:221–228.
- Galland, P., and V. E. A. Russo. 1984. Light and dark adaptation in *Phycomyces* phototropism. *J. Gen. Physiol.* 84:101–118.
- Hamilton, W. C. 1964. Statistics in Physical Science. The Ronald Press Company, New York.
- Hartmann, K. M. 1977. Aktionsspektrometrie. In Biophysik: Ein Lehrbuch. W. Hoppe, W. Lohmann, H. Markl, and H. Ziegler, editors. Springer-Verlag, Berlin, Heidelberg. 197–222. (English edition: 1983. Biophysics. Springer-Verlag, New York. 115–144.)
- Hertel, R. 1980. Phototropism in lower plants. In Photoreception and Sensory Transduction in Aneural Organisms. F. Lenci and G. Colombetti, editors. Plenum Press, New York and London. 89–105.
- Jesaitis, A. J. 1974. Linear dichroism and orientation of the *Phycomyces* photopigment. *J. Gen. Physiol.* 63:1–21.
- Lipson, E. D. 1975. White noise analysis of *Phycomyces* light growth response system. II. Extended intensity ranges. *Biophys. J.* 15:1013–1031.
- Lipson, E. D., and S. M. Block. 1983. Light and dark adaptation in *Phycomyces* light-growth response. *J. Gen. Physiol.* 81:845–859.
- Lipson, E. D., P. Galland, and J. A. Pollock. 1984. Blue light receptors in *Phycomyces* investigated by action spectroscopy, fluorescence lifetime spectroscopy, and two-dimensional gel electrophoresis. In Blue Light Effects in Biological Systems. H. Senger, editor. Springer-Verlag, Berlin, Heidelberg, New York. 228–236.
- Lipson, E. D., and D.-P. Häder. 1984. Video data acquisition for movement responses in individual organisms. *Photochem. Photobiol.* 39:437–442.
- Lipson, E. D., and D. T. Terasaka. 1981. Photogeotropism in *Phycomyces* double mutants. *Exp. Mycol.* 5:101–111.
- Löser, G., and E. Schäfer. 1980. Phototropism in *Phycomyces*: a photochromic sensor pigment? In The Blue Light Syndrome. H. Senger, editor. Springer-Verlag, Berlin, Heidelberg, New York. 244–250.
- Otto, M. K., M. Jayaram, M. Hamilton, and M. Delbrück. 1981. Replacement of riboflavin by an analogue in the blue-light photoreceptor of *Phycomyces*. *Proc. Natl. Acad. Sci. USA.* 78:266–269.
- Russo, V. E. A. 1980. Sensory transduction in phototropism: genetic and physiological analysis in *Phycomyces*. In Photoreception and Sensory Transduction in Aneural Organisms. F. Lenci and G. Colombetti, editors. Plenum Press, New York and London. 373–395.
- Russo, V. E. A., and P. Galland. 1980. Sensory physiology of *Phycomyces blakesleeianus*. *Struct. Bonding.* 41:71–110.
- Zankel, K. L., P. V. Burke, and M. Delbrück. 1967. Absorption and screening in *Phycomyces*. *J. Gen. Physiol.* 50:1893–1906.