Light and Dark Adaptation
in *Phycomyces* Phototropism

PAUL GALLAND and VINCENZO E. A. RUSSO

From the Max-Planck-Institut für Molekulare Genetik, Abteilung Trautner, D-1000 West Berlin 33, Federal Republic of Germany

ABSTRACT Light and dark adaptation of the phototropism of *Phycomyces* sporangiophores were analyzed in the intensity range of 10^{-7}–6 W·m^{-2}. The experiments were designed to test the validity of the Delbrück-Reichardt model of adaptation (Delbrück, M., and W. Reichardt, 1956, Cellular Mechanisms in Differentiation and Growth, 3–44), and the kinetics were measured by the phototropic delay method. We found that their model describes adequately only changes of the adaptation level after small, relatively short intensity changes. For dark adaptation, we found a biphasic decay with two time constants of $b_1 = 1$–2 min and $b_2 = 6.5$–10 min. The model fails for light adaptation, in which the level of adaptation can overshoot the actual intensity level before it relaxes to the new intensity. The light adaptation kinetics depend critically on the height of the applied pulse as well as the intensity range. Both these features are incompatible with the Delbrück-Reichardt model and indicate that light and dark adaptation are regulated by different mechanisms. The comparison of the dark adaptation kinetics with the time course of the dark growth response shows that *Phycomyces* has two adaptation mechanisms: an input adaptation, which operates for the range adjustment, and an output adaptation, which directly modulates the growth response. The analysis of four different types of behavioral mutants permitted a partial genetic dissection of the adaptation mechanism. The hypertropic strain L82 and mutants with defects in the *madA* gene have qualitatively the same adaptation behavior as the wild type; however, the adaptation constants are altered in these strains. Mutation of the *madB* gene leads to loss of the fast component of the dark adaptation kinetics and to overshooting of the light adaptation under conditions where the wild type does not overshoot. Another mutant with a defect in the *madC* gene shows abnormal behavior after steps up in light intensity. Since the *madB* and *madC* mutants have been associated with the receptor pigment, we infer that at least part of the adaptation process is mediated by the receptor pigment.

INTRODUCTION

Among microorganisms, the fungus *Phycomyces blakesleeanus* is particularly well suited for studying the phenomenon of adaptation. The unicellular fruiting body or sporangiophore, which grows at a rate of 2–3 mm/h, can undergo transient

changes of its growth rate in response to various stimuli, such as light, wind, ethylene, and closely placed barriers (Russo and Galland, 1980; Lipson, 1980). The growth responses to all of these stimuli are adaptive. To the physiologist, the responses to light are of particular interest, since the sporangiophore can adapt to an enormous intensity range from 10^{-9} to 10 W·m^{-2}. The response of the sporangiophore to unilateral light takes the form of positive phototropism. Symmetrically applied step or pulse changes of light intensity elicit transient changes of the growth rate, which can be either positive or negative, depending on an actual increase or decrease of intensity (Foster and Lipson, 1973). The magnitude of the transient growth response depends primarily on the subjective intensity, i.e., the ratio of the actual intensity to the intensity at which the sporangiophore was previously adapted. This requires an adjustment of the sensitivity to any new intensity in the range of 10^{-9} to 10 W·m^{-2}. The time course for these sensitivity changes represents the kinetics of adaptation. The molecular basis of the adaptation mechanism of *Phycomyces* is still unknown and the actual state of adaptation must be indirectly inferred by testing the sensitivity of the system.

Delbrück and Reichardt (1956) proposed a formal model for adaptation and the modulation of the growth rate, in which the level of adaptation A is described by the following differential equation:

$$dA/dt = (I - A)/b, \tag{1}$$

where I is the light intensity and b is the time constant of adaptation. In equilibrium, the adaptation level takes the value of the given intensity I. The growth rate is a function of the subjective intensity $i = I/A$ (Delbrück and Reichardt, 1956). The authors sought to describe with this model two different phenomena: (a) the kinetics of light and dark adaptation, i.e., the sensitivity changes of the system, and (b) the transient changes in the growth rate. In the very limited intensity and time domain for which they tested their model, it seemed adequate. We sought to test their model by probing into a much larger intensity range for dark as well as light adaptation.

Kinetics of light and dark adaptation have previously been measured by an indirect method, in which the actual level of adaptation after step or pulse changes is inferred from the reaction to short test pulses given at certain intervals (Delbrück and Reichardt, 1956; Lipson and Block, 1983). This method is laborious, since it requires the establishment of precise dose-response curves for the light growth response in different intensity ranges (Lipson and Block, 1983). In our work we used the simpler phototropic delay method (Bergman et al., 1969), in which the phototropic delay is a measure for the "memory", i.e., the adaptation state of the sporangiophore. The results obtained with this method are basically in agreement with the results of Lipson and Block (1983), who inferred the adaptation level with short test pulses. We find that light and dark adaptation are not symmetric processes, as implied by the Delbrück-Reichardt model; both processes seem to be mediated along different pathways.

We included in this study four types of mutants with abnormal phototropism to allow a preliminary genetic dissection of the adaptation pathway. Of particular

importance, we feel, is the observation that two mutants with abnormal photo-geotropic action spectra, *madB* and *madC* (Galland, 1983), show significant differences in light and dark adaptation. Because alterations of the action spectrum are most plausibly explained by defects of the photoreceptor, this finding suggests that adaptation is at least partially mediated by the receptor pigment.

MATERIALS AND METHODS

Strains

The strains used in this work are listed in Table I.

Culture Conditions

Strains were grown in shell vials (10 mm in diameter × 30 mm high) with PDACA medium (potato dextrose agar enriched with casein hydrolysate). PDACA contained 4%

TABLE I
Strains of Phycomyces blakesleeanus

Strain	Genotype	Reference
NRRL1555	Wild type (−)	Bergman et al. (1973)
C2	carA5 (−)	"
C21	madA7 (−)	"
C47	madA35 (−)	"
C109	madB101 (−)	"
C112	madB104 (−)	"
C148	carA5 madC119 (−)	"
L82	mad-702 (−)	Lipson et al. (1983)

NRRL1555 is the wild-type strain. All other strains were derived from NRRL1555 by nitrosoguanidine mutagenesis.

potato dextrose agar (Difco Laboratories, Inc., Detroit, MI), 1.5 mg casein hydrolysate (Merck, Sharp & Dohme, West Point, PA), and 50 μg vitamin B$_1$ (Merck, Sharp & Dohme) per milliliter. Vials were inoculated with 5–10 heat-shocked spores (48 °C for 10 min). The inoculated vials were kept in transparent plastic boxes in 90–100% humidity under white fluorescent light (Universal White F40T12/UW; Sylvania/GTE, Exeter, NH). The intensity of the fluorescent light was 0.9 W·m^{-2}; 44% of the intensity was broadband blue light (measured with a BG28 Schott Blau filter). 4- and 5-d-old cultures containing sporangiophores, which were in stage IVb (Bergman et al., 1969) and had grown 2.4–2.8 cm high as measured above the agar surface, were used for experiments.

Experimental Conditions

All experiments were done in a temperature-controlled darkroom (21 ± 0.5 °C) under red safelight. For measurements of growth response, phototropism, and avoidance response, a single shell vial with a single sporangiophore was placed in a Lucite box (Röhm GmbH, Darmstadt, Federal Republic of Germany [FRG]; dimensions 12 × 12 × 12 cm) with a wet paper towel at the bottom. The Lucite box was loosely closed with a plastic cover in order to avoid convection. In avoidance experiments, the boxes were covered with aluminum foil with a small opening through which a barrier could be inserted. The barrier (a glass microscope slide) was attached to a micromanipulator outside the box.

Sporangiophores were illuminated horizontally; bilateral illumination necessary for pre-adaptation was achieved by placing a small mirror near the sporangiophore, opposite to the light source and outside the Lucite box. Depending on the intensity range, sporangiophores were adapted bilaterally for 1 (above 10^{-3} $W \cdot m^{-2}$) or 2 h (below 10^{-3} $W \cdot m^{-2}$). Unilateral light was given by removing the mirror. The growth and the bending response were measured every 2 min with horizontal microscopes equipped with a goniometer device accurate to ± 1 deg. The microscope also contained a micrometer ocular (Leitz, Wetzlar, FRG; magnification 12×) accurate to ± 2 μm. Two experiments were always performed in parallel with two microscopes and two sporangiophores. All experiments were done on a heavy stone table.

Light Source

Sporangiophores were illuminated with Leitz Prado Universal projectors, with lenses of focal length 500 mm. Blue light was obtained with a broad blue filter (2-mm-thick Lucite, Röhm GmbH; symmetrical transmission peak at 450 nm; 0.1% transmission at 360 and 580 nm). For all illuminations, a heat-transparent stray filter (3-mm-thick; homemade) was present; 5-mm-thick heat filters were inside the projectors. Red safelight was obtained with red Lucite (2-mm-thick, type 501; Röhm GmbH), which had a broad transmission peak ranging from 600 to 800 nm with a 0.1% transmission at 560 nm. Neutral gray filters were obtained from Schott (Mainz, FRG). The fluence rate was measured with a calibrated bolometer (Flächenbolometer after Kurlbaum, made by C. Lassen Berlin) kindly provided by Dr. B. Vennesland (Max-Planck-Institut, Forschungsstelle Vennesland, West Berlin, FRG).

RESULTS

Dark Adaptation

A convenient method for measuring the kinetics of adaptation is the so-called phototropic delay method (Bergman et al., 1969). The principle of the method is outlined in Fig. 1, *A* and *B*: a sporangiophore is adapted bilaterally to an

FIGURE 1. (*opposite*)   (*A*) Time course of the phototropic bending angle θ at constant light intensity. The sporangiophores were adapted bilaterally at a total intensity of $2I_0 = 6 \times 10^{-5}$ $W \cdot m^{-2}$ (broadband blue light). 10 min after the start of the recording, the sporangiophores were illuminated with unilateral light of the same total intensity. The phototropic delay is obtained by extrapolating back from the steady state bending to the zero response. (*B*) Time course of the phototropic bending angle after a step down in intensity. The sporangiophores were bilaterally adapted for 60 min at a total intensity of $2I_0 = 6$ $W \cdot m^{-2}$. 10 min after the start of the recording, the sporangiophores were illuminated with unilateral light of an intensity of 6×10^{-5} $W \cdot m^{-2}$. (*C*) Dark adaptation kinetics of the wild-type strain and the albino mutant C2 (*carA5*) (open diamonds and abscissa on the top of the figure). Sporangiophores were bilaterally adapted to broadband blue light at the intensities that are given by the highest points of each curve. At time $t = 0$, sporangiophores were unilaterally illuminated with the indicated intensities, and then phototropic delay was determined. In experiments above 10^{-3} $W \cdot m^{-2}$, the preadaptation period lasted 1 h; below that intensity, the preadaptation lasted 2 h. In this and the following figures, error bars represent the standard error of four experiments, unless indicated otherwise.

intensity I_0, and at time $t = 0$ it is exposed to unilateral light of intensity I. The phototropic delay is defined as the time at which the bending rate begins to be in steady state. Fig. 1, A and B, shows that the delay is a function of the ratio I_0/I: the greater the step down of light intensity, the longer the phototropic delay. The kinetics of dark adaptation of the wild-type strain NRRL1555 were determined with this method over an intensity range of 6×10^7 (Fig. 1 C). We find that the phototropic delay has biphasic kinetics, which can be fitted to the formula: $I = I_1 \exp(-t/b_1) + I_2 \exp(-t/b_2)$. Since the system has come into equilibrium with the given intensities before and after the step down, I reflects directly the level of adaptation. We therefore rewrite the empirical formula as:

$$A = A_1 \exp(-t/b_1) + A_2 \exp(-t/b_2). \tag{2}$$
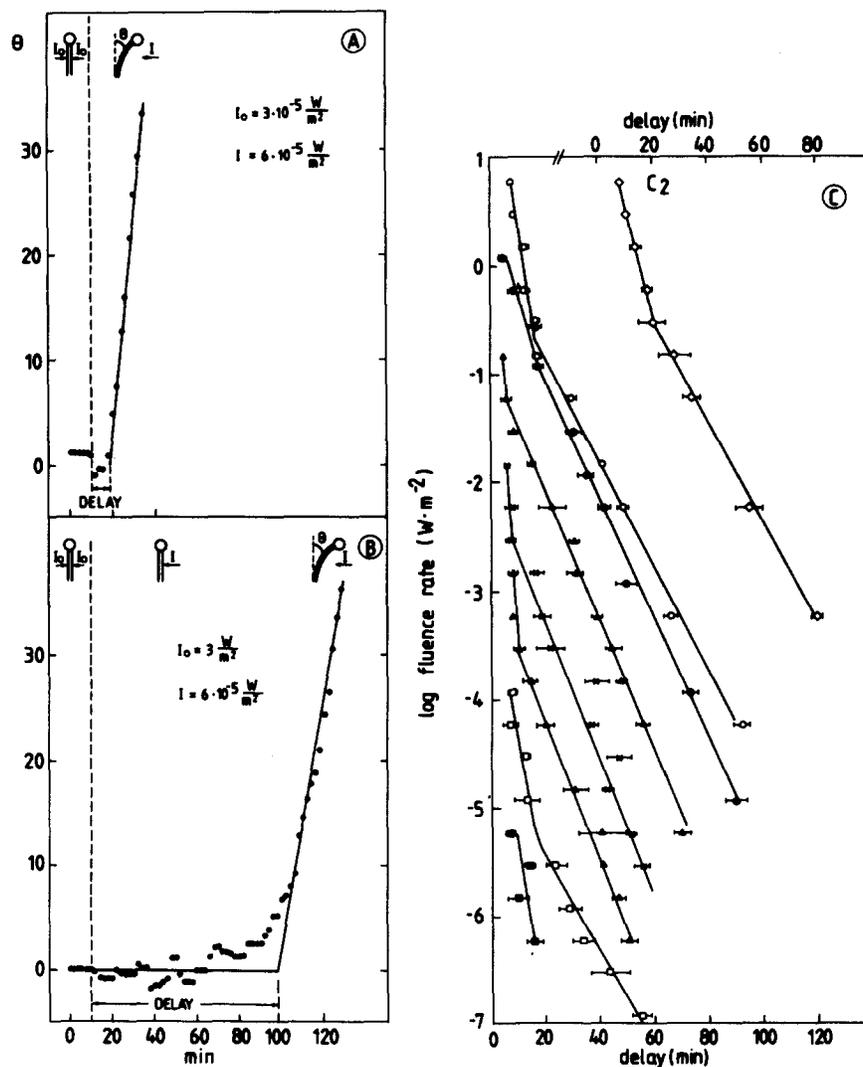
These kinetics of dark adaptation differ considerably from the predictions of the Delbrück-Reichardt model. The time constants b_1 and b_2, as well as the ratio $A_1/(A_1 + A_2)$, vary with the intensity range (Table II). In the high-intensity range (6 $W \cdot m^{-2}$) and the low-intensity range (6 $\times$ 10^{-6} $W \cdot m^{-2}$), b_1 and b_2 are significantly bigger than in the middle-intensity range (1.2 $\times$ 10^{-2} $W \cdot m^{-2}$). Furthermore, the state of adaptation in equilibrium depends on the absolute intensity; at 1.2 and 0.12 $W \cdot m^{-2}$, the ratio $A_1/(A_1 + A_2)$ is 0.54 and 0.57, respectively, while it is above 0.9 at 6 $W \cdot m^{-2}$ and 1.2 $\times$ 10^{-4} $W \cdot m^{-2}$. The β-carotene-lacking mutant C2 has the same biphasic dark adaptation kinetics as the wild type in the high-intensity range (Fig. 1 C); this shows that screening of this pigment does not

TABLE II

Dependence of Dark Adaptation Constants of Wild Type and Mutants Upon the Preadapting Intensity

Strain	Time constants of adaptation		Preadaptation intensity, I_0	$A_1/(A_1 + A_2)$*
	b_1	b_2		
	min	*min*	$W \cdot m^{-2}$	
C2 (*carA5*)	2	10	6	0.80
Wild type	1.7	9	6	0.91
"	4	8	1.2	0.54
"	2	7.5	1.5×10^{-1}	0.57
"	1	6.5	2.5×10^{-2}	0.76
"	1	7	2.5×10^{-3}	0.78
"	2	10	1.2×10^{-4}	0.98
"	2.3	—‡	6.0×10^{-6}	—‡
C21 (*madA7*)	4.5	13	6	0.90
"	4.5	12	1.2×10^{-1}	0.78
C47 (*madA35*)	4.5	13	6	0.90
C109 (*madB101*)	—	12	6	—
"	—	14	1.2	—
"	—	15	1.2×10^{-1}	—
C112 (*madB104*)	—	12	6	—
L82 (*mad-702*)	0.9	6	6	0.9

* A_1 and A_2 from Eq. 2 were determined graphically from Fig. 1.

‡ Only the fast phase of the adaptation kinetics was determined (Fig. 1).

introduce artifacts in our method. An important parameter in these experiments is the bending rate of the sporangiophores, which is a function of the absolute light intensity (Foster and Lipson, 1973; Russo, 1980). We wanted to know whether or not the bending rate also depended on the intensity of the pretreatment light or on the magnitude of the step down. Fig. 2A shows the bending rate for the experiments shown in Fig. 1 C. The bending rate does not depend on the intensity of the pretreatment light. Fig. 2B, which gives the pooled data of Fig. 2A, shows that the curve for the bending rate is biphasic, with a low-intensity component between 10^{-7} and 10^{-3} $W \cdot m^{-2}$ and a high-intensity component between 10^{-3} and 10 $W \cdot m^{-2}$. Previous curves of this type did not clearly show the two components (Foster and Lipson, 1973; Russo, 1980).

We tested the dark adaptation kinetics of four types of behavioral mutants. One type of mutant tested is the hypertropic strain L82, which has enhanced bending rates in phototropism, negative geotropism, and avoidance response (Lipson et al., 1983). Fig. 3A shows that L82 has faster dark adaptation kinetics than the wild type for both the fast and the slow component ($b_1 = 1$ min; $b_2 = 6$
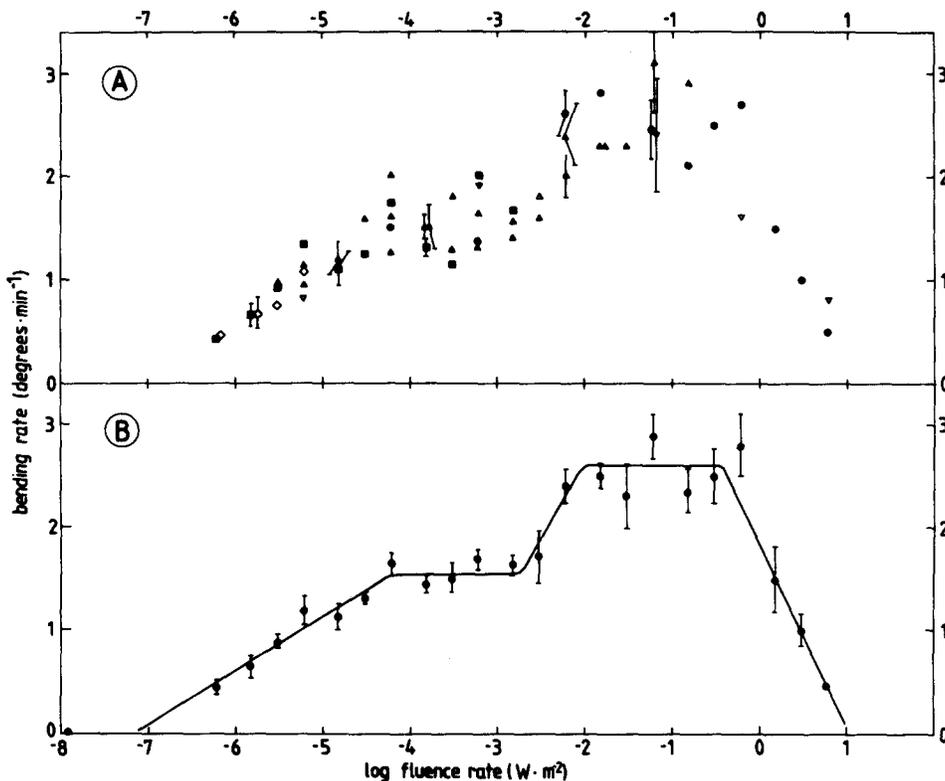

FIGURE 2. Dependence of bending rate of sporangiophores upon intensity of the unilateral light. The data are from experiments shown in Fig. 1C. (A) Symbols: ●, preadaptation to 6 W·m^{-2}; ▽, C2 (*carA5*), preadaptation to 6 W·m^{-2}; ▲, preadaptation to 1.2×10^{-1} W·m^{-2}; △, preadaptation to 1.2×10^{-2} W·m^{-2}; ■, preadaptation to 1.2×10^{-3} W·m^{-2}; ◇, preadaptation to 6×10^{-6} W·m^{-2}. The standard error is indicated for a few experiments. (B) Average of all experiments shown in A independently of the preadaptation. The bars indicate the standard error of the pooled experiments in A.

min). In addition, L82 has a complex intensity dependence as the dark adaptation kinetics in the middle-intensity range no longer follow the empirical formula (Eq. 2). The other mutants—affected in genes *madA*, *madB*, and *madC*— are so-called night-blind mutants, which have a raised phototropic threshold. Therefore, the adaptation kinetics could be tested only in the limited intensity range above the threshold. Fig. 3B shows the dark adaptation kinetics of *madA* strains

C21 and C47; both strains show the biphasic decay of adaptation characteristic of the wild type. The time constants (Table II) are, however, bigger than the ones of the wild type (b_1 = 4.5 min; b_2 = 13 min). Fig. 3C shows the kinetics of two *madB* strains, C109 and C112: both strains are lacking the first fast phase of
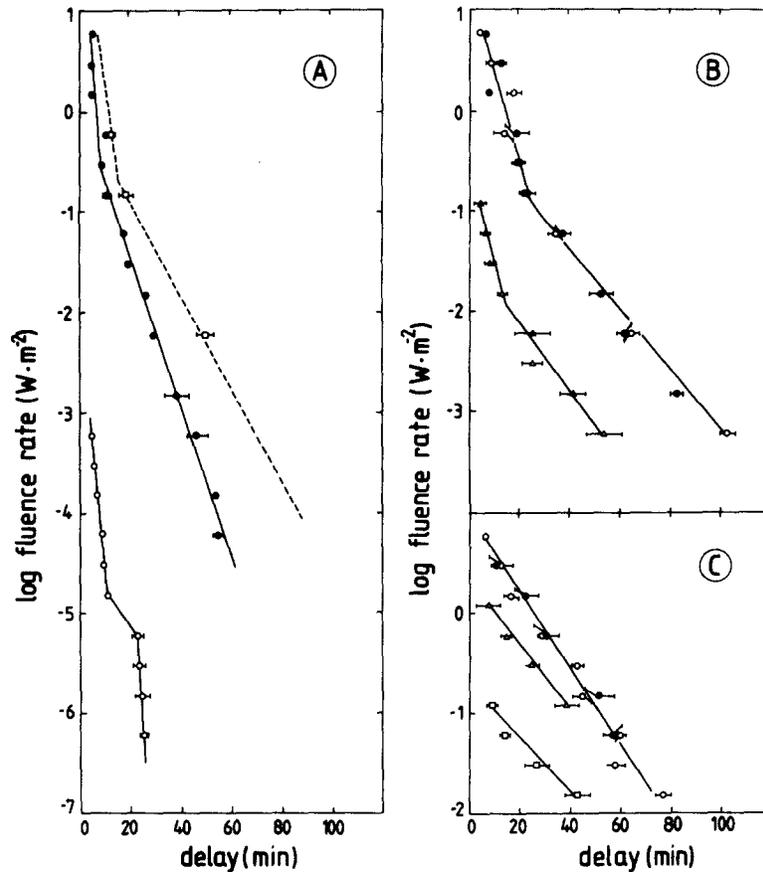

FIGURE 3. (*A*) Dark adaptation kinetics of the hypertropic mutant L82 (*mad702*). The experimental conditions were the same as in Fig. 1. The dashed line represents the wild-type control from Fig. 1 C; the open squares are control experiments of the wild type that were done at the same time as the experiments with L82. (*B*) Dark adaptation kinetics of *madA* mutants. The experimental conditions were the same as in Fig. 1. Symbols: O and △, C21 (*madA7*); ●, C47 (*madA35*). (*C*) Dark adaptation kinetics of *madB* mutants. The experimental conditions were the same as in Fig. 1. Symbols: △, O, and □, C109 (*madB101*); ●, C112 (*madB104*).

the biphasic curve. This gives genetic evidence that two different processes (under control of different genes) are involved in dark adaptation.

One of the aims of the Delbrück-Reichardt model was to describe the growth output as a function of the subjective intensity $i = I/A$. This implies that the

growth rate depends on the level of adaptation as long as $A \neq I$. In order to test this prediction, we performed the experiment shown in Fig. 4. Sporangiophores were adapted bilaterally to a given intensity I, and at time $t = 0$ the intensity was stepped down (Fig. 4B), while the illumination symmetry was maintained. The sporangiophores responded with a transient decrease of the growth rate, which
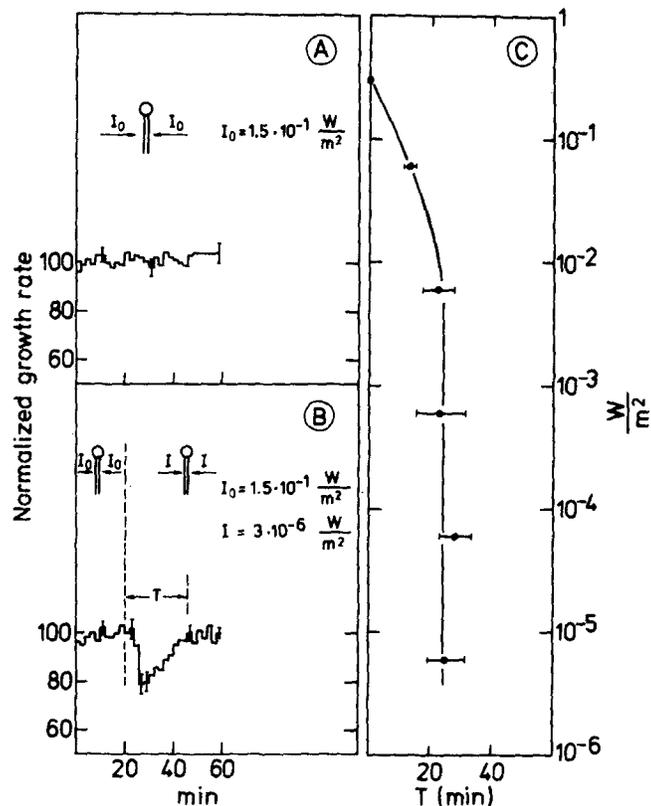

FIGURE 4. (A) Normalized growth rate of wild-type sporangiophores at constant light intensity $2I_0 = 3 \times 10^{-1}$ W·m^{-2} (broadband blue light). (B) Dark growth response of the wild type after a step down of light (at 20 min). T is the time at which the growth rate returns to the baseline level. (C) Time T as a function of light intensity after a step down of light. The values are obtained from a series of experiments of the type presented in B. The sporangiophores were always pre-adapted at $2I_0 = 3 \times 10^{-1}$ W·m^{-2} and the step down was made to different light intensities, which are given on the ordinate. Preadaptation time was 60 min.

returns to normal after ~22 min. Fig. 4C shows the time T, which is the duration of this dark growth response, as a function of the step down. We found that T is independent of the step down for values of I/I_0 below 1/50. This result is in contrast to the behavior of the phototropic delay, which increases with increasing steps down (Fig. 1). The return of the growth response must therefore be

regulated independently of the subjective intensity i and at a different site in the signal transduction chain.

Light Adaptation

We studied light adaptation with two different protocols for the phototropic delay to step as well as pulse stimuli. Fig. 5 shows the phototropic delay as a function of the step up in light intensity. Sporangiophores were adapted to three different intensity levels: 3×10^{-6}, 3×10^{-5}, and 3×10^{-2} W·m^{-2}. For all preadaptation intensities, the delay saturates at ~22 min. One complication in
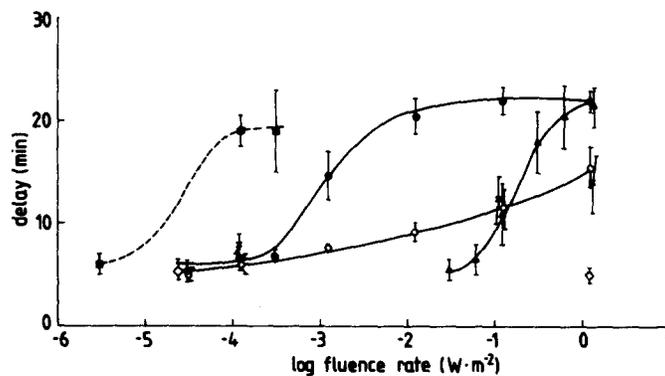

FIGURE 5. Phototropic delay of wild-type NRRL1555 after steps up of intensity of different magnitudes. Sporangiophores were adapted to 3×10^{-6} W·m^{-2} (■), 3 $\times 10^{-5}$ W·m^{-2} (●), and 3×10^{-2} W·m^{-2} (▲), and at time 0 the unilateral light was stepped up to the indicated intensities. In the experiments indicated by the symbols O and X, sporangiophores were bilaterally adapted to an intensity of 3×10^{-5} W· m^{-2}, and at time 0 the light was stepped up and a barrier was placed 0.6 mm away from the sporangiophore at the same time. Open circles: the barrier was placed opposite to the light source and unilateral light was given. Crosses: the barrier was given but the step up of light was done with bilateral light. The open diamonds indicate control experiments, in which the delay of the avoidance response was measured at the indicated intensities without changing the intensity of the bilateral illumination.

this type of experiment is caused by the transient light growth response, which is elicited by the step up of intensity. A transient saturation of the growth output during unilateral illumination should retard the onset of phototropism as long as the saturation is maintained, because phototropism is produced by differential growth rates at the sites proximal and distal to the actinic light. In order to find out to what extent the observed delay was caused by the adaptation mechanism or simply by the saturation of the growth output, the following control experiment was done. Sporangiophores were adapted to 2×10^{-5} W·m^{-2} and, at the time when the intensity was stepped up, a barrier was placed near the sporangiophore on the side opposite to the light source. The avoidance response to the barrier has a delay of 5 min that is independent of the light intensity as long as

the intensity is kept constant. This is shown by the control experiment in Fig. 5 (open diamonds); even in darkness, the delay of the avoidance response is 5 min (data not shown). If the phototropic delay of 22 min that is observed after a saturating step up with unilateral light were due to saturation of the growth response, the avoidance response would also show a 22-min delay when a saturating step up is given at the same time. The experiments in Fig. 5 (open circles and X's) show, however, that the tropic delay in the presence of a barrier, combined with a step up of intensity, is always below the delay found with a light step up only. This holds true whether or not the step up of light intensity was
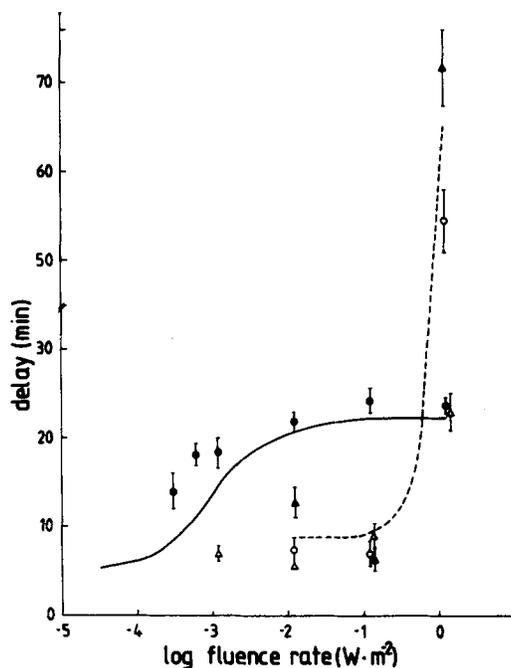

FIGURE 6.   Phototropic delay of three behavioral mutants as a function of a step up in light intensity. Sporangiophores were adapted bilaterally at 3×10^{-5} W·m^{-2}, and at time $t = 0$ unilateral light of the indicated intensities was given. Symbols: ●, C21 (*madA7*); ○, C109 (*madB101*); ▲, C112 (*madB104*); △, C148 (*madC119*). Solid line: wild type from Fig. 5. Dotted line: *madB* mutants.

given unilaterally (Fig. 5, open circles) or bilaterally (Fig. 5, X's). These two control experiments show that the phototropic delay after large steps up of unilateral light cannot be attributed exclusively to saturation of the growth output; the delay must therefore also be caused by the adaptation output. Why then is the delay of the avoidance response in these experiments not exactly 5 min but instead increases with increasing steps up? We believe that this is due to partial saturation of the growth output when the steps up become very large.

Behavioral mutants with defects in the genes *madA*, *madB*, and *madC* were also tested for the phototropic delay after steps up of unilateral light (Fig. 6). All

strains were bilaterally adapted to an intensity of 2.5×10^{-5} W·m^{-2}, an intensity that is below their respective phototropic threshold (Bergman et al., 1973). The *madA* mutant C21 behaves like wild type. The *madB* mutants C109 and C112 are, however, completely different; for steps up to 2.5 W·m^{-2}, they have an extra-long delay of 54 (C109) and 71 min (C112), respectively. The *madC* mutant C148 differs also from the wild type in that the delay does not increase very much from 1.5×10^{-3} to 1.5×10^{-1} W·m^{-2}. The extremely long delay of the *madB* mutants cannot be attributed to an abnormally long saturation of the growth response, because the response of the strain C109 to saturating steps up is even less than that of the wild type and returns to the baseline level after 30 min (Russo, 1980).
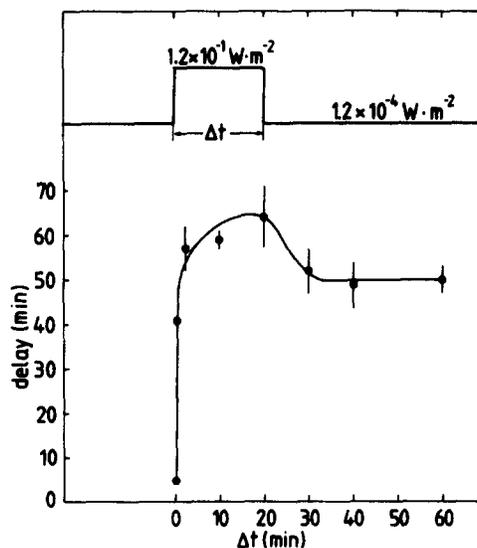

FIGURE 7. Phototropic delay of the wild type after a pulse of light. Sporangiophores were adapted bilaterally to an intensity of 1.2×10^{-4} W·m^{-2} for 90 min and a bilateral light pulse of 1.2×10^{-1} W·m^{-2} was given for variable durations, Δt. After the pulse, unilateral light of 1.2×10^{-4} W·m^{-2} was given and the phototropic delay was determined as a function of the pulse duration, Δt.

Step-up experiments of the type shown in Figs. 5 and 6 give information about the time when the adaptation level reaches the new level of light intensity, but they do not represent the actual kinetics of light adaptation. For that purpose, we used a procedure in which short, symmetrically applied light pulses of various durations are given. The phototropic delay after the pulse is a measure of the new intensity the adaptation level reached during the pulse.

Figs. 7 and 8 show that the dependence of the phototropic delay on pulse height and pulse width is a very complex one. The actual time course of the delay depends on the pulse height and also on the intensity range. For a relative pulse height of 10^3, the delay increases very quickly to a maximum of 60 min
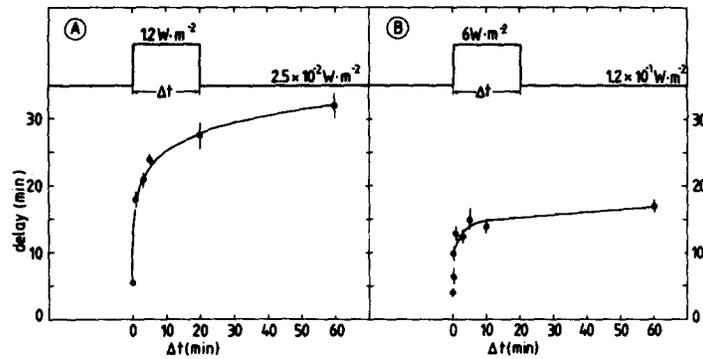
FIGURE 8. Phototropic delay of the wild type after a pulse of light. The phototropic delay is shown as a function of the pulse duration, Δt. (A) Sporangiophores were adapted bilaterally to 2.5 $\times$ 10^{-2} W·m^{-2} and a pulse of 1.2 $\times$ 10^{-1} W·m^{-2} was given. (B) Sporangiophores were adapted bilaterally to 1.2 $\times$ 10^{-1} W·m^{-2} and a pulse of 6 W·m^{-2} was given.

before it relaxes to a stable plateau value of 40 min. Pulses of height 50 reach the plateau value without overshooting the mark; the final delay time is dependent on the intensity range. The maximum delay is 32 min at 2.5 $\times$ 10^{-2} W·m^{-2} (Fig. 8A) and 17 min at 1.2 $\times$ 10^{-1} W·m^{-2} (Fig. 8B). The phenomenon of the overshoot and the dependence of the maximal delay on the intensity range are not predicted by the Delbrück-Reichardt model.

Pulse experiments in the high-intensity range were performed also with strains C21 and C109 for small pulses, which were 50 times above the adaptation intensity (Fig. 9). The *madB* mutant C109 shows an overshoot and reaches a plateau after 40 min. The *madA* mutant C21 behaves qualitatively like the wild
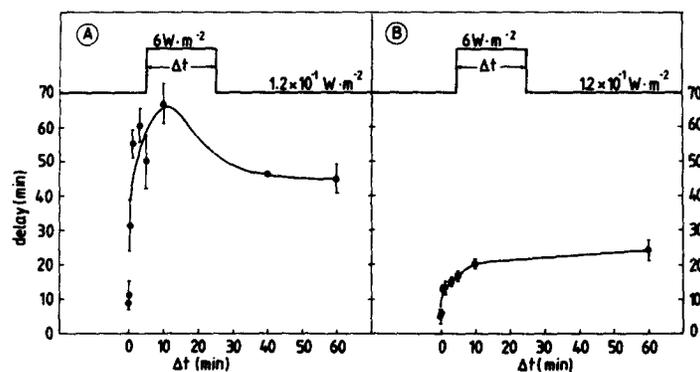
FIGURE 9. Phototropic delay of C109 (*madB101*) (A) and C21 (*madA7*) (B) after a pulse of light. Sporangiophores were adapted bilaterally to 1.2 $\times$ 10^{-1} W·m^{-2} and a pulse of 6 W·m^{-2} was given. The unilateral light after the pulse was 1.2 $\times$ 10^{-1} W·m^{-2} and the phototropic delay was measured as a function of the pulse length, Δt.

type and reaches a plateau after 10 min without showing the overshoot.

The *madC* mutant C148 has a peculiar adaptation defect in phototropism. Fig. 10 shows the phototropic response of C148 at 10^{-2} W·m^{-2} (filled circles). The *madC* mutant bends only 4 deg and then adapts to the unilateral light. However, when the same intensity is given after adaptation at 10^{-5} W·m^{-2}, the sporangiophore bends at least 12 deg (open circles). The total amount of bending depends on the magnitude of the step up (data not shown). Because of this phototropic defect of the *madC* mutant, dark adaptation kinetics like those in Fig. 1 could not be measured.
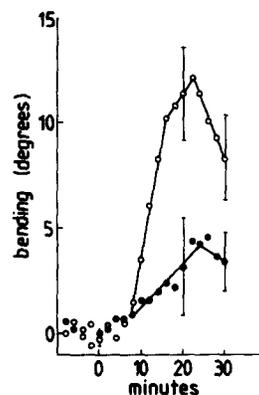

FIGURE 10.   Phototropic bending of mutant C148 (*madC119*). Filled circles: sporangiophores were adapted bilaterally to 1.2 × 10^{-2} W·m^{-2} for 90 min and were then exposed to unilateral light of the same intensity. Open circles: sporangiophores were adapted bilaterally to 1.2 × 10^{-5} W·m^{-2} for 90 min and were then exposed to unilateral light of 1.2 × 10^{-2} W·m^{-2}. The error bars indicate the standard error of eight experiments.

DISCUSSION

Delbrück and Reichardt tested their model of light and dark adaptation of *Phycomyces* sporangiophores for relatively small intensity changes and a narrow time range of 30 min. They proposed a first-order reaction for dark adaptation and found a time constant of $b = 3.8$ min for the first 10 min of the kinetics. We tested dark adaptation over a much larger intensity range (10^{-7}–6 W·m^{-2}) for steps down of the order of 2–10^6. Over this range, the dark adaptation kinetics are incompatible with the simple first-order reaction given by Delbrück and Reichardt. The kinetics of dark adaptation are biphasic and are described empirically by Eq. 2. This equation holds over the entire tested intensity range. For the light growth reaction, Lipson and Block (1983) found a biphasic decay only in the high-intensity range (10 W·m^{-2}), but a monophasic decay at 10^{-2} W· m^{-2}. They found a time constant of $b = 6$ min, while the time constants for the slow phase in our kinetics ranged from 6.5 to 9 min. Part of this discrepancy might be due to the differences in the assay system, since the authors had to infer the level of adaptation indirectly from the light growth responses of test

pulses. However, part of it may be caused by differences in the growth conditions prior to the actual experiment; we found that the light intensity at which the strains grow influences the kinetics of adaptation (Galland and Russo, 1984).

One complication in the dark adaptation experiments is the relatively long delay time of some 90 min, when the system undergoes large step changes of the order of 10^5–10^6. Since the growth rate of a sporangiophore is 2–3 mm/h, the 2-mm-long growing zone (strictly speaking, the external cell wall) is replaced during this period approximately one to two times. The implications of this fact for the dark adaptation are not entirely clear, however, since it is not known whether or not the receptor pigment grows out of the growing zone in the same way the cell wall does. The biphasic nature of the dark adaptation kinetics should in any case be independent of this complication, since the change from the fast to the slow phase occurs before 20 min, i.e., a time when approximately only a third of the growing zone (cell wall) could be replaced. The biphasic kinetics suggest that two different processes are involved in dark adaptation; this conclusion is also supported by the fact that the two *madB* mutants C109 and C112 are both missing the fast component.

The model of Delbrück and Reichardt attempts to explain with one formalism two different phenomena, namely changes in the level of adaptation (adaptation output) and transient changes of the growth rate (growth output). In this formalism, the growth output reduces to a function of the subjective intensity $i = I/A$. This implies that the growth rate depends upon i as long as $A \neq I$. However, the experiment shown in Fig. 4, combined with the dark adaptation kinetics in Fig. 1, shows that the return of the growth rate is independent of the actual state of adaptation. Delbrück and Reichardt made very clear the necessary distinction between adaptation output and growth output and they were also aware that the growth output can reach saturation before the adaptation output. Their model, however, would predict that the dark growth response to large steps down should stay (negatively) saturated as long as $A > I$, which is, however, not the case. Indeed, we confirmed that the growth output to large steps down becomes saturated (not shown in Fig. 4), but found that it has itself adaptive properties. We therefore redefine the distinction between adaptation output and growth output that was made by Delbrück and Reichardt, and distinguish between input adaptation (i.e., the range adjustment mechanism), output adaptation (i.e., habituation or modulation of the growth response; Ortega and Gamow, 1970), and finally the growth output itself.

The Delbrück-Reichardt model treats light and dark adaptation as symmetric processes and makes some clear predictions about the reactions to steps up of light intensity. The solution of Eq. 1 for steps up gives:

$$A = A_0 + (I - A_0) [1 - \exp(-t/b)], \qquad (3)$$

where A_0 is the preadaptation intensity and I is the intensity after the step up. The time required for A to reach the new intensity level depends mainly on the ratio of t to b and is almost independent of $(I - A_0)$; i.e., the height of the step up, if one assumes that the adaptation mechanism cannot resolve a difference $(I - A)$ smaller than 5% of $(I - A_0)$. This assumption is justified, because the

phototropic delay for step-up experiments in which $I = 2A_0$ is indistinguishable from experiments in which $I = A_0$. Assuming the time constant of adaptation of $b = 4.9$ min given by Delbrück and Reichardt, one should predict that after large steps up ($I \gg 2A_0$), the adaptation would reach nearly 96% of the new intensity level in 15 min; for $I = 2A_0$, the adaptation would reach 97.8% of the new intensity level in 15 min. Thus, the phototropic delay should be independent of the magnitude of the step up; this holds true whether or not two time constants are involved. We found, however, that the phototropic delay depends on the magnitude of the step up in the range from 2.5×10^{-5} to 2.5×10^{-2} W·m^{-2}, where it saturates at 22 min (Fig. 5). This could either mean that in light adaptation the time constant changes with the magnitude of the step up, or else that the actual adaptation level overshoots the new intensity value before it returns (see below).

The existence of a causal relation between the phototropic delay after a step up and the overshoot of the adaptation kinetics is supported by the observation that the *madB* mutant C109 has an extra-long delay of 54 min after a step up of 10^5 and that it has at the same time an overshoot in the light adaptation kinetics under conditions where the wild type has none (Fig. 9). By combining a step up of light intensity with an avoidance response, we were able to show that the phototropic delay is not due to a saturation of the growth output (Fig. 5); this is concluded because no avoidance response should ensue in the case of a saturation of the growth output. The fact that an avoidance response is observed after the double stimulus means that the observed phototropic delay is due to input adaptation. Our results are in agreement with the finding that an avoidance response can still be elicited immediately after a strong light stimulus when the light growth response is at its maximum (Ortega and Gamow, 1970).

In order to determine the actual kinetics of light adaptation, we used a method similar to that of Delbrück and Reichardt. In this procedure, one gives short pulses and infers the new adaptation level by either test pulses and measurement of the light growth response or, in our case, the phototropic delay. One can then calculate from the delay and the known phototropic dark adaptation kinetics (Fig. 1) the apparent level of adaptation reached during the pulse. In this way one can "reconstruct" the kinetics of adaptation for a step up of light intensity. This method was used by Lipson and Block (1983) for test pulses of a relative height of 10^3: they found that the adaptation first overshoots the new intensity level before it relaxes to this level. For pulses of the same height, we also found an overshoot. In Figs. 7–9, however, we did not show the calculated level of adaptation but the actual phototropic delay after the pulse. The reason for this is that the calculation of the apparent level of adaptation is usually done with dark adaptation curves (Fig. 1), which were obtained after steady state conditions and not after pulses. One cannot a priori assume that both kinetics will have identical properties. We calculated, however, the level of adaptation for Figs. 7–9 and indeed obtained an overshoot similar to the one found by Lipson and Block (data not shown). The light adaptation kinetics are further complicated by the fact that they also depend on the intensity range (Fig. 8). For pulse heights

of a factor of 50, the kinetics can be described by a first-order reaction as proposed by Delbrück and Reichardt. An increase in the intensity range causes an increase in the time constant. None of these complications can be explained in the framework of the Delbrück-Reichardt model.

A photochemical model that associates the kinetics of adaptation with those of the photoreceptor was proposed by Lipson (1975). In this model, the adaptation A is solely a function of p, the fraction of active photoreceptor. For the nonstationary state, the following equation was derived (Lipson, 1975):

$$dA/dt = k (I - A) (1 + A/I_c),$$ (4)

where $k = 1/b$ and I_c is the critical intensity at which half of the photoreceptor is bleached. I_c itself was not determined experimentally, but was derived theoretically in the context of white noise analysis (Lipson, 1975). In the normal-intensity range, i.e., below 0.6 $W \cdot m^{-2}$, where bleaching is negligible ($A \ll I_c$), Eq. 4 reduces to Eq. 1 of the Delbrück-Reichardt model and therefore predicts a monophasic exponential decay of adaptation. In the high-intensity range ($A > I_c$), the correction term ($1 + A/I_c$) cannot be neglected, and the solution of Eq. 4 given by Lipson and Block (1983) does predict a biphasic decay of A. However, our dark adaptation data show clearly biphasic decays at high as well as low intensity and therefore contradict the photochemical model of Lipson (1975). Furthermore, the phenomenon of the overshoot found in light adaptation is not predicted by the photochemical model.

One of our aims in this work was to undertake a preliminary genetic dissection of the adaptation mechanism. We found that the *madA* mutants C21 and C47 and the hypertropic mutant L82 are qualitatively similar to the wild type. Differences were found, however, in the time constants of dark adaptation. The *madB* mutants C109 and C112 are very different from the wild type in dark as well as light adaptation; the *madC* mutant C148 shows differences in light adaptation (Fig. 6), but dark adaptation could not be tested for technical reasons (see Fig. 10). The fact that the dark adaptation kinetics of *madB* mutants are lacking the fast phase shows that the slow and the fast phases belong to different molecular events, which are controlled by different genes. However, from the kinetics of the *madB* mutants alone, one cannot determine whether or not the fast process (b_1) was lost or became longer and therefore indistinguishable from the slow process (b_2). A clear assignment of a specific gene to the fast or slow adaptation process seems at the moment impossible; this is seen best from the fact that all four types of behavioral mutants cause an alteration of the adaptation constants. It seems that the adaptation machinery is a complex molecular structure that is under the control of many genes.

madB and *madC* mutants have photogeotropic action spectra that are very different from the wild type. These genes have therefore been associated with the receptor pigment (Galland, 1983). Because of their defects in adaptation, we conclude that the adaptation process is at least partially mediated by the receptor pigment. It is possible that part of the input adaptation takes place at the photoreceptor level and that other parts take place at a different site in the

transduction chain. A functional linkage between adaptation and the photoreceptor can explain how one mutation (*madB* or *madC*) can cause a threefold change in the phenotype, namely abnormal action spectra, altered adaptation kinetics, and a rise in threshold.

The functional relation between dark adaptation and threshold is the subject of the accompanying paper (Galland and Russo, 1984).

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