A Kinetic Model for Adaptation and the Light Responses of Phycomyces

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ABSTRACT A kinetic model is described consisting of two sequential first order processes connected by two parallel reaction pathways, one of which is light-catalyzed. A change in light flux changes the rate constant of the light-dependent process, whereupon the levels of two chemical intermediaries readjust. The model's output duplicates all the main features of the cell's light-growth and dark-growth responses except their latent periods. An asymmetric modification of the model reproduces the two types of phototropic inversion discovered by Reichardt and Varjú and by Dennison. Simple exponential equations describe these responses of the model, as well as the theoretical course of its light and dark adaptation. It is concluded that adaptation in Phycomyces consist in the photocatalytic adjustment of the level of a metabolic reservoir.

The Phycomyces cell is a unitary photoreceptive-reactive system in which the responses to light are modulations of the cell's continuous rate of elongation. This relatively undifferentiated system exhibits a full range of characteristic photoreceptor phenomena, such as "on" and "off" responses to changed levels of illumination, intensity discrimination, and adaptation; as far as is known, no significant bioelectric changes enter into these processes. The basic facts about the light responses were established by pioneer investigations in Blauw's laboratory in the Netherlands, culminating in a particularly important analysis by Oort (1932). More recently, these responses have been systematically analyzed from a biophysical point of view by Delbrück and his associates; their two most relevant papers are those by Delbrück and Reichardt (1956) and Reichardt and Varjú (1958).

My special interest was aroused by Dennison's (1965) discovery that a cell bending steadily under unilateral irradiation shows a temporary reversal in the direction of bending (phototropic inversion) when the light beam's intensity is reduced. It was already known that in such an experiment a similar transient inversion occurs when the light intensity is increased. This paradoxically symmetric behavior, wherein either a step-up or a step-down in intensity evokes the same phasic response, must have a specific kinetic basis.
It therefore seemed that a model able to accommodate these newer phototropic phenomena and also the more general features of the responses to light would have passed an unusually critical test. Such a model should help clarify our understanding of the mooted nature of adaptation.

THE BASIC MODEL

My model is intermediate between the ideas of the investigators in the Netherlands and the more abstract conceptions of Delbrück's group, and owes much to both. It is built on the concrete idea, dictated by measurements of growth during bending (Castle, 1961 a; 1962), that the cell's response is conditioned by the supply of a material, $M$, transported into the growth zone from below and used stoichiometrically in growth. In the model, $M$ is transformed into a product $P$. Light is taken to catalyze this conversion, but there is a second reaction pathway from $M$ to $P$ that is independent of light. This must be so, for the steady-state growth rate is essentially the same either in the light or in the dark. The existence of these two pathways is found to be decisive for the inversions. Finally, the rate of transformation of $P$ is assumed to constitute the instantaneous growth rate.

The net result of this scheme is to introduce two reservoirs of intermediates, $M$ and $P$, into the reaction sequence; the kinetics of response and of adaptation are governed by relatively slow changes in them rather than in a photoproduc-t. Changes in the rate constant of the light reaction, either up or down, can be considered instantaneous and the mathematics is simplified.

We shall represent these sequential processes as if they were first order irreversible chemical reactions having one light-catalyzed step, and shall ignore any complication of enzyme-substrate complex formation. The following scheme results for the case of symmetrical irradiation:

\[ e \rightarrow M \xrightarrow{(k' + k)} [M] \xrightarrow{j} [P] \rightarrow \text{wall formation} \]

where $c$ is the constant rate of supply of $M$, $[M]$ and $[P]$ are the respective concentrations of these materials, $k'$ and $k$ are the light-dependent and the light-independent rate constants of the first reaction, and $j$ is the rate constant of the second reaction. The growth velocity is defined as $V = jP$.

In the steady-state the following equations hold:

\[ \frac{dM}{dt} = c - (k' + k)M = 0; \quad \frac{dP}{dt} (k' + k)M - jP = 0 \quad (1) \]

where $k'$ may be thought to express the concentration (or activity) of an enzyme determined by the light intensity. Solution of equations (1) gives the following for the temporal changes of $M$ and $P$ in any experiment in which $k'$
has taken on a new value:

\[ M = \frac{c}{k' + k} - \left( \frac{c - (k' + k)\rho M}{k' + k} \right) e^{-(k' + k)t} \]  \hspace{1cm} (2)

\[ P = \frac{c}{j} - \left( \frac{c - (k' + k)\rho M}{j - (k' + k)} \right) e^{-(k' + k)t} + \left[ P - \frac{c - (k' + k)\rho M}{j - (k' + k)} \right] e^{-jt} \]  \hspace{1cm} (3)

where \( \rho M \) and \( \rho P \) are the initial concentrations of these substances. If, as is usually the case, the experiment starts from a steady-state and we are interested chiefly in the growth velocity itself, equation (3) is multiplied by \( j \), and becomes:

\[ V = \frac{c - (k' + k)\rho M}{j - (k' + k)} \left( e^{-(k' + k)t} - e^{-jt} \right) \]  \hspace{1cm} (4)

For most of the present calculations it is convenient to start from a standard steady-state condition where \( k' = 0.01 \), \( k = 0.01 \), \( j = 0.1 \), and \( c = 1 \). This gives \( \rho M = 50 \), \( \rho P = 10 \), \( V = jP = 1 \). Then if, for example, the light intensity rises so that \( k' \rightarrow 0.04 \), the time course of the normalized growth velocity from equation (4) becomes the simple exponential expression

\[ V = 1 + 3(e^{-0.05t} - e^{-0.1t}) \]  \hspace{1cm} (5)

Certain reservations should be noted. (a) The ratio \( k'/k \) defines the proportions of \( M \) converted to \( P \) through the light and dark channels, and the ratio \( (k' + k)/j \) sets the relative sizes of the reservoirs of \( M \) and of \( P \). Values of these constants are arbitrarily chosen to approximate the facts of the growth responses. (b) \( k' \) is assumed to increase with the light intensity, but certainly not linearly; this relation, which is probably logarithmic, is here left unspecified. (c) Since the cell in fact grows away from its ultimate source of metabolites at variable speeds, it may not be valid to assign \( c \) an always constant value. But to introduce \( c \) as another dependent variable complicates the analysis and invokes knowledge of such unknowns as the real capacity of the \( M \) reservoir and the linear speed of the \( c \) transport mechanism; hence for simplicity \( c \) is taken constant. (d) First order differential equations cannot express such subtleties of the real growth response as its variable latent period and the initial sigmoid course of the acceleration of growth; the model does not reproduce these features.

Three typical responses of the cell and of the model are compared in Fig. 1. A step-up in light intensity (top horizontal row of Fig. 1) is equivalent to a rise in \( k' \) for the model; \( M \) declines exponentially to a new steady-state level, while \( P \) rises to a maximum and then decays to its fixed steady-state level; the resulting extra growth is equal to the drop in the steady-state level of \( M \).
Following a step-down (middle horizontal row), \( M \) rises exponentially while \( P \) passes through a minimum and rises from below to its normal level; the net loss of growth is equal to the steady-state gain in \( M \). The response to a temporary pulse-up (bottom horizontal row) is an abbreviated combination of the foregoing processes with no net change in the integrated growth output.

**Figure 1.** Three basic light responses of the cell (left vertical column of three graphs), and of the model (middle and right vertical columns). The abscissa of all graphs is time; \( V \), normalized growth velocity; \( I \), light intensity; \( k' \), the light-dependent rate constant of the model; \( M \) and \( P \), the respective levels of these substances in the model. Top horizontal row of graphs, responses to a lasting step-up in light intensity (or in \( k' \)); middle horizontal row, responses to a lasting step-down; bottom horizontal row, responses to a brief pulse-up. The responses of the cell are generalized from various published data and are diagrammatic; \( V \), \( M \), and \( P \) for the model are calculated from equations derived in the text. The scale of the model's \( t \)-axis is arbitrary.

It is clear that, except for the latent period, the major aspects of the cell's responses are duplicated by the model.

With increasing light doses, the magnitude of the response in both cell and model increases toward a limit. Delbrück and Reichardt found that the cell's response was in its middle range linear with \( \log I \). We cannot make a strict comparison with the model because for it the relation between \( k' \) and \( I \) is
not defined. However, Fig. 2 A depicts the model’s responses to a graded series of increases in \( k' \). The magnitude of a response is logically measured by the extra growth produced, which for the step-up case is \( \int_0^t (V_1 - 1) \, dt \). More directly, it is also the difference between the initial and final steady-state levels of \( M \). Fig. 2 B shows the model’s increasing response, at a progressively diminishing rate, with increase in \( k' \).

A final relation of interest, but again one which cannot be rigorously tested, is the reciprocity between time and intensity for short exposure times. This is a well established fact for the cell, and is commonly thought of as an expression of the Bunsen-Roscoe law. It is equally true, however, that if such a photochemically based relation holds, it must be able to express itself, undistorted, through such intermediates as \( M \) and \( P \). Fig. 3 shows the model’s responses to three increases in \( k' \) for brief times reciprocally shortened in proportion to the increment in \( k' \). In this case where the experiment produces no net gain in total growth, we may consider that the area under the positive phase of each curve measures the response. Thus the response is \( \int_0^t (V - 1) \, dt \), where \( t \) is the time when each response curve crosses the line \( V = 1 \). As shown in Fig. 3, \( R \) obtained by integration of the three curves over this interval is sensibly constant. One can therefore at least say that the model’s behavior is compatible with the reciprocity principle.
THE ASYMMETRIC MODEL

Phototropic bending is an asymmetric response wherein a small difference between growth velocities across the cell causes curvature. The time course of bending is commonly measured as increase in the total angle of deviation from the cell's original direction; for unilateral visible light of fixed intensity, such bending is at a steady rate of several degrees per minute and is essentially independent of the light intensity. The inversion phenomena occur when a steadily bending cell receives either a sudden sufficient increase or decrease in light flux: in each case bending ceases, reverses, and ultimately recovers. Fig. 5 (top) reproduces Dennison's measurements of both types of inversion. But in one case the cell's total growth is temporarily augmented, in the other diminished; nevertheless, the difference across the cell goes through a comparable temporal cycle.

Incorporation into the basic model of an additional fact and an additional assumption equips it to give the two types of inversion. The additional assumption is that through the cell's optical mechanism unilateral light maintains \( k' \) constantly higher on the far side of the cell than on the near side.
The additional fact is that during bending more growth takes place on the far side than on the near side, while at the same time the total growth is limited by a fixed rate of supply of $M$.

We shall denote the far and near sides of the cell by the subscripts two and one respectively. Then for the case of unilateral irradiation the asymmetric scheme is:

\[
\begin{align*}
&\text{far wall formation} \\
&\text{near wall formation} \\
&\text{direction of light infall}
\end{align*}
\]

The symbols $c$, $k'$, $k$, $j$, $M$, and $P$ have their previous meaning; $a$ is a factor greater than one that denotes the photochemical advantage of the far half; $c'$ expresses the lateral transport (or "leakage") of $M$ that supports the consistently faster growth of the far side. By definition, $V_2 = jP_2$, $V_1 = jP_1$.

Then if $c > c' > 0$, the following inequalities exist during steady phototropic bending:

\[
\begin{align*}
c + c' &> c - c' & M_2 &< M_1 \\
k' + k &> k' + k & P_2 &> P_1 \\
V_2 &> V_1 
\end{align*}
\]

It is particularly noteworthy that the $M_1$ reservoir is at a higher steady-state level than the $M_2$ reservoir; this fact is the basis of the inversions.

Formulation leads to equations for the two sides of the cell that parallel equations (1), (2), and (3) above but which contain the new terms $c'$ and $a$.

Thus equation (4) becomes the pair

\[
\begin{align*}
V_2 &= (c + c') - j \left( \frac{(c + c') - (k' + k) M_2}{j - (k' + k)} \right) \left( e^{-(k' + k)t} - e^{-jt} \right) \\
V_1 &= (c - c') - j \left( \frac{(c - c') - (k' + k) M_1}{j - (k' + k)} \right) \left( e^{-(k' + k)t} - e^{-jt} \right)
\end{align*}
\]

We again take a standard steady-state condition for the purposes of practical calculation. As before, $k' = 0.01$, $k = 0.01$, $j = 0.1$, and $c = 1$. The two halves of a bending cell differ by about 12% in their growth velocity (Castle, 1965),
so \( c' = 0.06 \); both \( c \) and \( c' \) are assumed constant throughout the responses.\(^1\) Finally we take \( a = 2 \), which implies that light doubles \( k' \) on the far side of the cell.\(^2\) Then in this standard steady state \( \sigma M_1 = 47.0, \sigma M_2 = 35.3, \sigma P_1 = 9.4, \sigma P_2 = 10.6, V_1 = 0.94, V_2 = 1.06. \)

The model's rendition of the two inversions is shown graphically in Fig. 4. When \( k' \) changes at time zero, \( V_1 \) and \( V_2 \), which are calculated from equations (6 a, 6 b), follow different time courses, crossing twice before reaching their steady states. For the period when \( V_1 > V_2 \), bending is reversed in direction. We may define the normalized bending rate as \( B = (V_2 - V_1)/2c' \). The top curves of Fig. 4 show the time course of \( B \), negative bending being diagonally shaded. Although the time scales are different for the step-up and step-down responses, the minima in the \( B \) curves occur at about the same absolute time; this is also known to be true for the cell in the case of inversions of moderate size.

The actual measurements on a bending cell are bend angle as a function of

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\(^1\) The facts of bending show that the process symbolized by \( c' \) is real. We describe it neutrally as lateral transport of \( M \). There is no evidence that this is an active process driven by light. As the model is framed, \( M \) moves down a gradient.

\(^2\) The decisive assumption that \( a = 2 \) cannot be theoretically justified because the theory of the lens effect is not fully understood (Dennison, 1965; Castle, 1965). It is clear that \( a \) must be greater than one, and the model also works well if \( a = 3 \). Conceivably the magnitude of \( a \) is related to the locally high light intensity at the central area of the cell's far half, which Reichardt and Varjá calculated to be more than twice the intensity incident on the face of the cell.
time. Fig. 5 (top) gives Dennison's unsmoothed data for the two types of inversion. Fig. 5 (bottom) shows the accumulated bending vs. time for the model's two responses, where \( \phi = \int B \, dt \), calculated as \( \int (V_2 - V_1)/2e' \, dt \). These particular responses of the cell and of the model would not be expected to be identical, since Dennison's light intensity changed fourfold and for the model \( k' \) was arbitrarily changed tenfold. The only adjustment made in this comparison was to give the graphs of Fig. 5 the same initial slope. The similarity is striking.

**Figure 5.** Top, Dennison's measurements on *Phycomyces* of a step-up inversion (left), and of a step-down inversion (right); \( \phi \), bend angle; \( I \), relative light intensity. Bottom, comparable plots for the model obtained by integration of the \( B \)-curves of Fig. 4; both \( \phi \) and \( t \) are arbitrary here.

**DISCUSSION**

The imitative success of the model strongly implies that adaptation resides in photocatalytic adjustment of the level of a metabolic reservoir. In such "flow" systems, light acts as a valve facilitating the supply or preparation of a material used in growth. When the light intensity changes, this valve, which is \( k' \), can be taken to change its aperture instantaneously. The steady-state levels of two chemical intermediaries, \( M \) and \( P \), which are respectively before and after the valve, then readjust themselves; the time course of their output is the observed growth response. The level of the \( P \) reservoir always returns to the same value, whereas the steady-state level of the \( M \) reservoir varies

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*There is even a suggestion in Dennison's step-up data of an increase in bending rate before the decline and reversal, an effect which is prominent in the response of the model (Fig. 4, left, top) but which does not appear in Reichardt and Varjo's plots.*
with the openness of the valve $k'$: therein is the mechanism of what Delbrück terms the "range adjustment." Thus stationary levels of adaptation of the system as a whole are uniquely set by the light intensity.

On the other hand, the transition between steady states is dominated by relatively slow changes in the active masses of the reaction chain's intermediaries; these constitute the material link between the photochemical part of the system and the growth output—what Delbrück has abstractly called the system's "subjective intensity." It is well established in visual systems that light and dark adaptation are also not quantitatively accounted for solely by changes in the photoreceptive pigment.

Measurement of the progress of light and dark adaptation in a flow system is beset by both experimental and conceptual difficulties. But since the model's inner mechanisms are known, we can readily deduce its theoretical course of adaptation; the simplest case is that following a step in intensity. For this purpose, the state of adaptation may be taken as the system's ability to respond, which concerns the momentary condition of its reservoirs of $M$ and $P$.

Consider light adaptation in the basic model, starting from the standard conditions stated on p. 927. Let $k'$ increase from 0.01 to 0.04 at time zero. From equations (2) and (3),

$$M = 20 + 30e^{-0.04t}$$  \hspace{2cm} (7)

$$P = 10 + 30(e^{-0.064} - e^{-0.11})$$ \hspace{2cm} (8)

These are plotted in the top right hand graph of Fig. 1. Now suppose that at any time $t$ we turn on and keep on an infinitely bright light so that in effect $k' \to \infty$; this instantaneously drains the $M$ reservoir. The system's response to this test is to yield an extra amount of growth. The extra growth is the algebraic sum of the deviations of the levels in the $M$ and $P$ reservoirs at time $t$ from their ultimate steady-state levels; in this case the latter are $M_\infty = 0$, $P_\infty = 10$. Thus the theoretical response at time $t$ tested by a sustained infinite step-up in light intensity is

$$R_t = (M_t - M_\infty) + (P_t - P_\infty)$$
Substituting for $M_t$ and $P_t$ from equations (7) and (8) gives

$$R_t = 20 + 60e^{-0.06t} - 30e^{-0.01t}$$

for the theoretical course of light adaptation, expressed in terms of response, as the model adjusts from $k' = 0.01$ to $k' = 0.04$ (Fig. 6).

The reverse change when $k'$ falls from 0.04 to 0.01 (Fig. 1, middle right hand graph) gives, by parallel reasoning and the same test, the model's theoretical course of dark adaptation as

$$R_t = 50 - 37.5e^{-0.02t} + 7.5e^{-0.01t}$$

which is also plotted in Fig. 6. Each curve is slightly sigmoid, and dark adaptation has, as is known for *Phycomyces*, a greater time constant. Indeed, the behavior of the model in adaptation is nowhere inconsistent with present empirical knowledge of adaptation in *Phycomyces*.

Cohen and Delbrück (1959), who conceived adaptation less inclusively, have discussed the suggestion by Jaffe that there may be local differences in adaptation within the cell. From the present position we must affirm this idea, which is central to the operation of the asymmetric model. When such a local difference between the $M_2$ and $M_1$ reservoirs has been established by prior irradiation from a particular direction, the phototropic inversion following intensity increase given from any direction whatever is initially in that preestablished plane; this is also true for *Phycomyces* (Castle, 1961 b). Furthermore, the fact, puzzling to several authors, that the steadily bending cell does not "adapt" to unilateral irradiation and cease bending, is exemplified by the permanent asymmetric steady state maintained by factor $a$ of the model.

Lastly, I do confess that $M$ and $P$ are hypothetical substances, but I am sustained by the belief that a pattern of behavior is more the product of a system than of its named constituents.

REFERENCES


