

## GROWTH DISTRIBUTION IN THE LIGHT-GROWTH RESPONSE OF PHYCOMYCES

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### ABSTRACT

Elongation of sporangiophores marked with numerous starch grains was photographically recorded in the steady state and during the light-growth response when the rate is more than doubled. From these records the spatial distribution of growth within the cell's growth zone was derived.

Stimulation by a single saturating flash of light speeds growth proportionally in all parts of the growing zone, maintaining the same pattern of growth distribution as in the steady state. This finding implies that light is absorbed and acts locally throughout the length of the cell's growth zone. Cohen and Delbrück's proposal of a partial spatial separation of light reception and growth is discussed.

The mature *Phycomyces* sporangiophore responds to an increase in the intensity of illumination with a delayed, transient increase in growth rate, the so called light-growth response, measurable either in the rate of the cell's elongation or its axial twist. Given a cell suitably dark-adapted and an adequate light stimulus, the growth rate may briefly more than double.

Both growth and sensitivity to light are confined to a region a few millimeters long immediately below the terminal sporangium. This growing zone is continuously self-propagated upward, and maintains its position and extent relative to the cell's terminus. Growth is the sum of a graded series of growth increments characteristically distributed over the length of the growing zone; this distribution can be mapped by markers applied to the outside of the cell membrane. The following study examines the patterns of growth in the steady state and during the enhancement of growth by light.

### *Material and Methods*

Mature (stage 4b) sporangiophores of *Phycomyces blakesleeanus*<sup>1</sup> were marked by dusting with starch grains, grown vertically under diffuse light from above in a moist chamber, and periodically photographed much as described previously (Castle, 1958). A favorably marked cell may have from 10 to 30 grains visible on the profile of its growing zone; unless present in great excess or in gross clumps these do not depress

<sup>1</sup> "Minus" strain kindly supplied by M. Delbrück.

or distort growth. After the cell had reached a steady state of growth in the moist chamber, serial photomicrographs were taken at 1 or 2 minute intervals in strong red light through a horizontal microscope with a 32 mm. microtessar objective and a microibso attachment for the Leica camera; resulting magnification on the film was 6.7 times. After development for maximum contrast, the positions of individual markers were measured on the film under a binocular microscope with ocular micrometer scale. Distortion by the optical system was found negligible except at the extreme edges of the field photographed.

Owing to spiral growth, markers within the growth zone not only change in longitudinal position but also revolve about the cell's long axis, most markedly near the

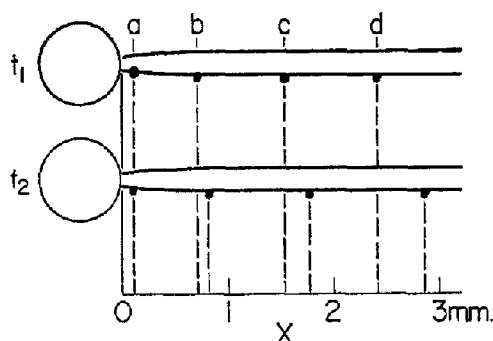


FIG. 1. Two views of a growing sporangiophore, represented horizontally, with starch grain markers  $a$ ,  $b$ ,  $c$ ,  $d$  attached. Positions are measured along the  $x$  axis from the base of the sporangium. Growth in the interval  $t_1$  to  $t_2$  displaces each marker a distance  $\Delta x$ , except marker  $a$  which is outside (above) the growth zone. The spiral component of growth is not shown.

top of the growth zone. Markers rotating sufficiently to pass into transit across the front or back surfaces of the cell are lost from view by the present method, but such loss is minimized by photographic records spaced only 1 minute apart. The method has the real advantage that information is recorded simultaneously for markers throughout the growth zone. The results that follow are wholly based on measurements of the longitudinal component of growth.

Growth in the steady state took place at a temperature of  $26^\circ \pm 1^\circ\text{C}$ . under white light diffused from above of about 0.2 foot-candle intensity. The light-growth response was induced by a single superimposed exposure for 20 seconds to unilateral white light (water-cooled) of 180 foot-candles, obtained by brief withdrawal of the Corning signal red filter from the horizontal light beam used for photographic recording. Phototropic curvatures did not follow this asymmetrical exposure, so it may be assumed that the cell's photosensitive system is symmetrically saturated ("phototropic indifference"; Castle, 1931). Serial photography continued following the light stimulus, and the maximum response was taken for the 1 minute interval about 5 minutes following the onset of the stimulus.

Measurements on the film give the instantaneous positions of markers on the

surface of the growing wall, all distances being measured from the sporangium base along the cell's long axis. Fig. 1 illustrates on this basis how a series of markers within the growth zone behave with time:  $x$ , the distance from the sporangium base, increases slowly for markers near the top of the growth zone, progressively faster for those lower down, and at a constant maximal rate for those at and below the bottom of the growth zone. Subtraction of corresponding measurements on successive pictures gives the velocity of longitudinal motion of each marker,  $\Delta x/\Delta t$ . For pictures 1 minute apart, the position of the marker during this interval may with only small error be taken as the average.

For a series of markers, a plot of  $\Delta x/\Delta t$  against  $x$  gives a *rate of displacement* curve that contains the basic information on the momentary distribution of growth through-

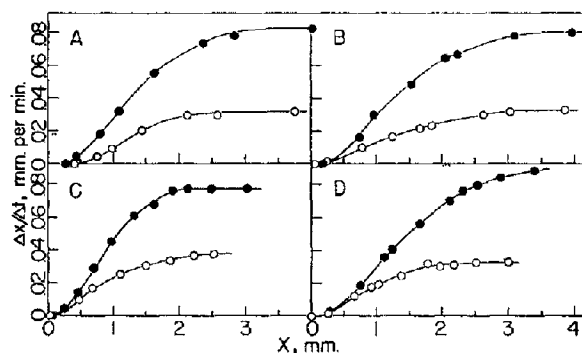


FIG. 2. Rate of displacement curves for four separate cells, A, B, C, D. Abscissa: distance below the sporangium. Open circles: growth in the steady state; solid circles: growth at the maximum of the light response.

out the growing zone. An explicit measure and representation of growth rates at different points is secured by differentiation of the rate of displacement curve with respect to position, and by plotting  $d(\Delta x/\Delta t)/dx$  as a function of  $x$ . This differential is Erickson's *relative elemental growth rate* (Erickson and Sax, 1956; for an independent analysis see also Cohen and Delbrück, 1958). The results below are presented in terms of these two plots.

#### RESULTS AND DISCUSSION

Fig. 2 shows rate of displacement curves for four representative cells chosen for presentation because their growth zones were well marked, especially in the critical upper region. In each case the *lower* curve shows growth in the steady state, the *upper* growth at the maximum of the light response about 5 minutes after stimulation. Each plotted point relates to a separate marker, its ordinate being simply the difference between two measurements of position. In some cases the reliability of points on the steady state curves is increased by averaging over a 3 or 4 minute interval; such averaging is not possible during the response to light because the rate changes rapidly with

time. Points on the light response curves are intrinsically more reliable, however, because the measured change in position of a marker per unit time is about twice that in the steady state.

The curves drawn through the points are fitted by eye. They first start to rise from the abscissa not at the origin but a few tenths of a millimeter from it, showing the presence of a short non-growing region immediately below the sporangium. Thereafter the curves run an asymmetrically sigmoid course, levelling off to a limiting ordinate value at the end of the growth zone. Inspection of the steady state and light response curves for any one cell shows that they have very much the same shape. If this is so, they should superimpose when the lower (steady state) curve is multiplied by a factor that raises

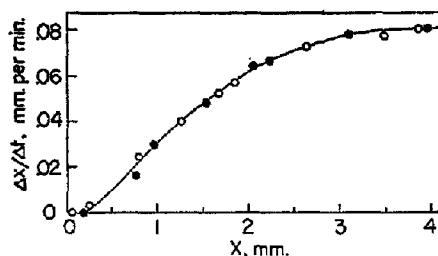


FIG. 3. Rate of displacement curve for cell *B* of Fig. 2. Solid circles: at the maximum of the light response; open circles: points for the steady state multiplied by the factor 2.43 (see text). A single curve fits the growth distributions before and during the light response.

its limiting ordinate value to that of the upper (light response) curve. The results of such multiplication are shown in Fig. 3. In three of the four cells (*A*, *B*, and *C*) the superimposition is satisfactory. In one case (*D*) it is not; this particular cell, unlike the other three, exhibited a marked increase in length of the growing zone following stimulation by light. We conclude that, within the limitations of measurement here used, the *shape* of the rate of displacement curve is essentially the same before and at the maximum of the light response.

Fig. 4 presents the same results in terms of relative elemental growth rate. As stated above, this is an explicit measure of growth rate; it expresses the per cent increase of an infinitely short segment of the cell per unit time, and as plotted depicts the distribution of growth rates along the cell, both in the steady state and after exposure to light. Ordinates in Fig. 4 were obtained by taking the slopes of the curves drawn in Fig. 2. Since these curves were fitted by eye and since differentiation is a sensitive operation, the plots of Fig. 4 will exaggerate any errors made in drawing the rate of displacement curves. Nevertheless the similarity of the steady state and light response curves for

any one cell is apparent. Basically the spatial pattern of growth appears to be unchanged by the light stimulus, the effect of which is to speed growth at every point within the growth zone proportionally.

This result is not surprising. It implies that light is absorbed throughout the length of the growth zone and that its effect on growth is local. Significant transport of a product of light action up or down the cell seems excluded by the short time available: about 5 minutes from the onset of the stimulus to the peak of the growth response. Furthermore, a separate study of the

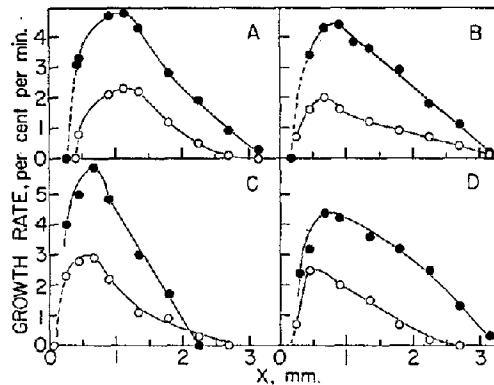


FIG. 4. Growth distributions of cells *A*, *B*, *C*, *D* obtained by differentiation of curves in Fig. 2. Ordinate: relative elemental growth rate. Open circles: in the steady state; solid circles: at the maximum of the light response. The shapes of the curves are not reliable in detail, but show that the response to light is a generally proportional increase in growth rate throughout the growing region. Dashed lines are extrapolations or uncertainties at the extreme top of the growth zone.

rising and falling phases of the light-growth response (2 minutes before and after the maximum) shows no evidence of disproportionate or out-of-phase response by parts of the growth zone.

Cohen and Delbrück (1958), however, from detailed marker experiments have concluded that the cell's region of fastest growth shows no response to light, and that light sensitivity is confined to a mid-region of the growth zone less than half its whole length. If this were true, the maximal relative elemental growth rate should be independent of light, and the shape of the growth distribution during the light response should be radically different from that in the steady state. Figs. 2, 3, and 4 above show that these conditions are not met. We conclude that in the light-growth response of dark-adapted cells to a saturating flash of light the spatial separation of light sensitivity and growth proposed by Cohen and Delbrück does not exist.

The cause of this disagreement is obscure. The present measurements

were made on cells in a truly steady-state exposed once to a strong light stimulus. Cohen and Delbrück used light stimuli repeated at regular intervals of 5 minutes; under such periodic stimulation there is never an approximation to a steady state. That these authors found no response to light at the region of maximal growth rate might indicate depletion of a photoreceptive substance supplied from below the growth zone; this could be adequately present throughout the zone in the steady state condition of our experiments, or regional differences in its concentration might be masked by our use of a saturating flash of light.

Continuous upward self-displacement of the growing zone clearly requires a supporting upward transport of material from below. If, under the conditions used by Cohen and Delbrück, light depressed the supply of a substance participating in the light-growth response, its depletion should be most marked *farthest* from the source, namely, at the *top* of the growth zone. This they found to be the case, though their further conclusion that the bottom third of the growth zone also shows no response to light is incompatible with any simple transport hypothesis.

Our own results give no evidence of regional "uncoupling" of light reception from growth, and support the simpler view that the light-growth response of a cell in the steady state is the sum of extra growth induced by the absorption and action of light throughout the growing zone. As regards the phototropic response to prolonged asymmetric illumination, itself the result of asymmetrically induced growth, there is accumulating evidence that it has temporal and spatial features distinct from those of the light-growth response (Cohen and Delbrück, 1959). The separation of transient effects of light from its persistent action in phototropism should help clarify the behavior of this cell in response to light.

#### REFERENCES

- Castle, E. S., *Bot. Gaz.*, 1931, **91**, 206.  
Castle, E. S., *J. Gen. Physiol.*, 1958, **41**, 913.  
Cohen, R., and Delbrück, M., *J. Cell. and Comp. Physiol.*, 1958, in press.  
Cohen, R., and Delbrück, M., *J. Gen. Physiol.*, 1959, **42**, 677.  
Erickson, R. O., and Sax, K. B., *Proc. Am. Phil. Soc.*, 1956, **100**, 487.