Plant Physiol. (1967) 42, 1665-1672

Low Energy Effects of Light on Growth and Pigment Content in a Yellow-in-the-Dark Mutant of Chlamydomonas reinhardi[1]

John Terborgh[2], Kaye V. Ladd, and Guy C. McLeod
Tyco Laboratories, Inc., Waltham, Massachusetts 02154

Received June 2, 1967.

Summary. The y-2 mutant of *Chlamydomonas reinhardi* differs from the wild type in being unable to synthesize chlorophyll in the dark and in a requirement for catalytic amounts of light for organotrophic growth. Light-grown y-2 cells given acetate are capable of the equivalent of 9 to 10 divisions when placed in darkness. Cultures adapt gradually to dim white or monochromatic light and after 8 to 10 generations assume a steady state with respect to growth and pigment content.

Two energetically distinct light reactions promote the growth of y-2 on acetate. A low energy requirement is satisfied at about 0.1 $\mu w/cm^2$ of white light which results in a growth rate of 0.5 log unit per day. A high energy response, which saturates at 2000 $\mu w/cm^2$ and a growth rate of 0.9 log unit per day, is probably attributable to net photosynthesis. An action spectrum for the low energy growth response contains a broad major peak in the blue between 462 and 502 nm and a minor peak in the far-red between 700 and 736 nm. All intermediate wavelengths have low but positive activity. The action spectrum was investigated with y-2 cultures that were grown for many generations under steady-state conditions in growth-limiting monochromatic light. Many wavelengths resulted in a selection pressure that strongly favored a strain of green-in-the dark cells that usually appeared after 5 to 8 generations of light-limited growth. Under the low light intensity of these experiments (0.15 ± 0.05 $\mu w/cm^2$) the green strain was much richer in chlorophyll than y-2 and divided more rapidly with the consequence that y-2 was generally replaced in the course of a few generations. Consideration of the results led to the conclusion that both chlorophyll and carotenoids act as photoreceptors in the low energy growth response of y-2.

At least 3 types of metabolism make possible the presence of algae in natural situations that are penetrated by sub-compensating quantities of light. A) The temporary survival of phytoplankton at depths below the euphotic zone may be greatly prolonged by the action of small amounts of photosynthetically active light. *Dunaliella tertiolecta* totally labeled with ^{14}C lost radioactivity through respiration much less rapidly when given the equivalent of 1 ft-c of light than in complete darkness (3). B) A number of algae are facultative organotrophs (e.g., species of *Chlorella, Scenedesmus, Euglena* and *Chlamydomonas*) and can propagate indefinitely in total darkness when provided with a suitable energy source (2). C) Certain unicellular green algae have the capacity to assimilate exogenous organic compounds and to divide 1 or more times in absolute darkness, but then cease to divide further unless given minute quantities of light. It is this special effect of light on cell division that is the subject of our investigations.

In studying a strain of *Chlorella vulgaris* which failed to grow in the dark on glucose, Killam and Myers (10) found that the cells divided at a moderate rate when the cultures were subjected to a daily optical density reading in a colorimeter that contained a red filter. Karlander and Krauss (9) later revealed that the energy requirement for this effect in *Chlorella vulgaris* saturates at 0.8 $\mu w/cm^2$ of white light. Their action spectrum shows sharp peaks in effectiveness of wavelengths near 425 nm and 575 nm, and was interpreted as evidence that the photoreceptor is a cytochrome. Our results with a very similar effect in a mutant of *Chlamydomonas reinhardi* differ in several important respects with regard to the action spectrum and lead to a dissimilar conclusion.

Materials and Methods

The experiments employed a yellow-in-the dark mutant of the green flagellate, *Chlamydomonas*

[1] This work was supported by contracts NOw-64-0343-C and NOw-65-0538-C from the Department of the Navy, Bureau of Naval Weapons.

[2] Present address: Department of Botany, University of Maryland, College Park, Maryland 20740.

reinhardi. This mutant, designated as y-2, was originally isolated in the laboratory of Prof. R. P. Levine who kindly supplied us with the culture used in this work. Y-2 differs from the wild type of *C. reinhardi* principally in being unable to synthesize chlorophyll in darkness (5) and in failing to grow indefinitely in the dark on acetate (4). In the light the performance of the mutant with regard to growth and chlorophyll content is indistinguishable from that of the wild type (5). Both forms grow well as phototrophs or, when supplied with acetate, as photoorganotrophs.

Experimental cultures as well as those used for inocula were grown axenically in cotton-stoppered 250 ml or 500 ml Erlenmeyer flasks on a rotary shaker. The medium of Sueoka (15) was used throughout with or without the addition of 0.2% sodium acetate. This medium contains phosphate buffer to minimize the change in pH on addition of acetate. Increase in cell number was exponential under the conditions used until the cell density exceeded 5×10^6/ml. Experimental cultures were always initiated with exponentially growing cells and diluted with fresh medium often enough to maintain population densities below 3×10^6 cells/ml.

The protocol of the experiments required continuous illumination of 100 ml or 200 ml cultures under constant conditions for periods of several days to 2 weeks or longer. In order that a number of wavelengths or light intensities could be investigated simultaneously in this fashion, a large rotary shaker was modified as follows. A pair of clear lucite plates was erected over the shaker on a superstructure of aluminum rods that were bolted onto the rotating platform. From the lower lucite plate was slung a battery of 20-watt daylight fluorescent tubes that provided approximately 800 ft-c at the surface of the upper lucite plate. A 1-inch air space between the plates greatly reduced the heating of the upper plate by the lamps. Several rows of culture flasks could be arranged on the upper plate and held in place by crossbars clamped to the superstructure that supported the whole device. The water temperature in the flasks was normally 28 ± 1° but occasional slight deviations from this level during the long-term experiments were unavoidable.

In 1 series of experiments cultures were illuminated with white light of various energies over a range of nearly 10^5-fold. This was accomplished by using Bausch and Lomb 2″ × 2″ neutral density filters. To achieve the desired intensities 1 or more of these were taped to the bottoms of flasks that had been otherwise thoroughly wrapped in electrician's tape. Aluminum foil caps prevented stray light from entering through the cotton stoppers. The cultures inside thus received light only from below through the filters.

A second series of experiments called for very low energy irradiations with monochromatic light. The technique employed was similar to that used in the white light experiments except that the flasks were placed in a light-tight wooden box that contained 8 isolated compartments. Set into the bottom of each compartment was a square 2″ × 2″ hole that exactly held an interference filter. The filters were supplied by Baird-Atomic, Inc. (series B-2 and B-3) and had steep-sided transmission bands with one-half band widths of 15 to 30 nm. The transmission characteristics of the filters were determined spectrophotometrically and none were found to pass additional bands in the visible or at wavelengths shorter than 1.0 μ in the near IR. Since very small quantities of light were needed, the filters were covered with electrician's tape so that light was allowed to pass only through a small rectangular opening, usually less than 0.5 cm^2 in area. The light energies that reached flask bottom level after passing through the various filters and filter combinations were measured with an Eppley thermopile. In the action spectrum experiments the amount of energy reaching the cultures was regulated by varying the area of the tape-bordered openings on the filters through which light was allowed to pass.

Most of the experiments were carried through a number of serial transfers of the cultures. The quantity of cell suspension that was inoculated into fresh medium on each occasion depended on the expected cell density and growth rate of the culture being transferred. In this manner cell densities in all the cultures of a series were kept within the same range of 10^4 to 3×10^6 cells/ml. At the end of a run, normally after 2 to 4 days of growth, each culture was apportioned in the following manner. An appropriate amount, usually 2 to 10 ml, was used for inoculum to continue the experiment. Another 2 ml was set aside for hemacytometer counts of cell density. The remainder was then drawn through a glass fiber filter (Gelman) in order to harvest the cells. The filter, which normally contained about 10^8 cells, was then ground in 10 ml of 80% acetone in a homogenizer to extract the pigments. On filtration the homogenate yielded a clear extract that was subjected to spectrophotometric analysis. The optical density at 470 nm was taken as a measure of the carotenoids present and the amount of chlorophyll was computed from the absorption at 663 nm using the formula given by Bruinsma (1).

Results

Given strong light, 28°, and a medium containing acetate, the y-2 mutant of *C. reinhardi* increases in number at a maximum rate of 0.93 log 10 unit per day, corresponding to a generation time of 7 to 8 hours. In the absence of acetate the best phototrophic rate we obtained was only 0.53, though under the circumstances growth may have been limited by the rate of CO_2 diffusion into the flasks.

Preliminary experiments revealed that cell division in *C. reinhardi* quickly becomes synchronous when the cultures are given brief periodic exposures

to light. Even the small regular change in light intensity that resulted from turning on and off the overhead lights in the laboratory effectively synchronized cultures that received 800 ft-c of continuous illumination on an open shaker. Thus it became apparent that an investigation of the special effect of light on cell division would have to employ continuous low level irradiation. Otherwise, the results would be complicated by a tendency of intermittent irradiation to influence the timing of cell increase in an unpredictable manner. Some early experiments did indicate, however, that a few minutes' illumination per day sufficed to stimulate division and apparently could adequately substitute for the low intensity continuous irradiations used in these experiments.

Light-dependent Pigment Synthesis. When y-2 is given acetate and placed in the dark or in dim light, there is little initial change in the rate of cell increase though the amount of chlorophyll and carotenoid pigments in the cells begins to decline immediately (figs 1 and 2). The decrease in pigment content continues steadily for the equivalent of 8 or 9 generations. Since 2, 4 or 8 zoospores may be released in each asexual cycle, it is not completely accurate to refer to generations, though we shall continue to do so for the sake of convenience. Cultures that are maintained in continuous dim light for more than 8 or 9 generations attain a steady state with respect to chlorophyll and carotenoids. Once such a steady state has been established, there are no further changes in pigment concentration in the cells; the rate of pigment synthesis is exactly balanced by the increase in cell number. Unless there is selection for other cell types, there are no further pigment changes in the steady-state cultures for at least 40 generations, or as far as we have carried the experiments.

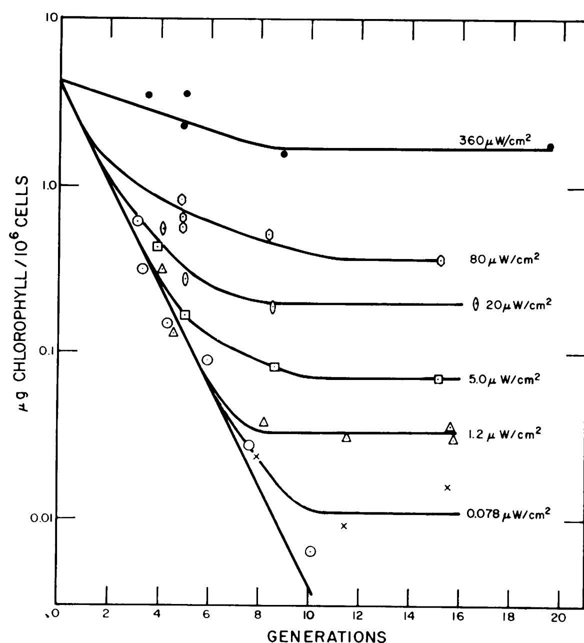

FIG. 1. Kinetics of the chlorophyll content of y-2 in darkness (circles) and in various intensities of white light. The starting material was grown in acetate medium in 4 mw/cm² of white light. Chlorophyll contents reached steady-state levels after 8 to 10 generations. The tangential line represents a 2-fold dilution of chlorophyll with each generation.

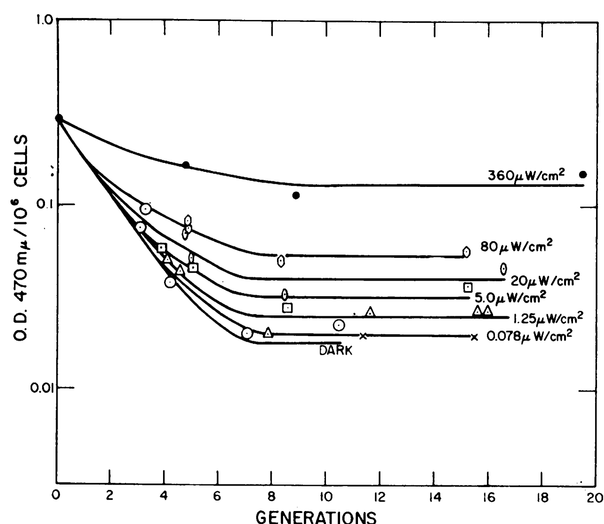

FIG. 2. Kinetics of the carotenoid content of y-2 in darkness and in various intensities of white light. The extracts were the same as those used for the chlorophyll determinations shown in figure 1. Steady states were attained after about 8 generations.

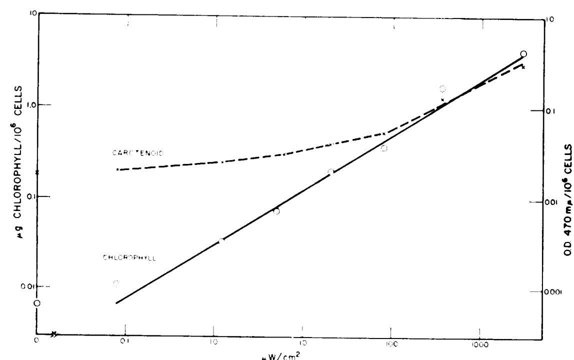

FIG. 3. Steady-state values for chlorophyll and carotenoid contents of y-2 cultures as a function of light intensity on a double logarithmic plot. The points were taken directly from the steady-states shown in figures 1 and 2.

The carotenoid and chlorophyll contents of steady-state cultures (hereafter meaning > 10 generations in dim light) showed different forms of dependence on light (fig 3). At the lowest intensities the carotenoid content was nearly independent of the energy received and about 7 % that of light grown cells. The remaining 93 % of the normal carotenoid complement is thus due to a light promoted synthesis which is most strong in the

range above 100 μw/cm^2 at levels that can be expected to exceed the compensation point.

In contrast, the logarithm of the chlorophyll content of steady-state cultures was a linear function of the logarithm of the incident light energy over the entire range studied of nearly 10^5 fold. At 0.08 μw/cm^2 the cells maintained only 0.2 % of the amount of chlorophyll that is normal in bright light. The data from cultures placed in total darkness fall closely along a line representing a 2-fold dilution of chlorophyll with each generation (fig 1), indicating that there is no detectable light-independent chlorophyll fraction.

Cell Division. A culture that is placed in total darkness continues to increase at nearly the maximum rate for about 6 generations. Divisions then become much less frequent and after about 5 days there is no further increase in cell number. Maximum dark growth yields the equivalent of 9, or rarely 10, generations, an increase of roughly 500 to 1000 fold. Cells that have ceased to divide are exceptionally large and conspicuously packed with starch granules.

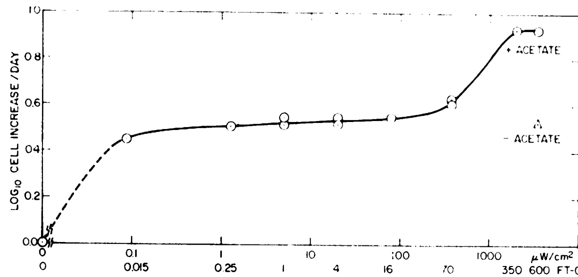

FIG. 4. The steady-state growth rate of y-2 as a function of the logarithm of the intensity of white fluorescent light. All cultures contained acetate except the one indicated. The data pertain to cultures that had been grown for a minimum of 12 generations under the specified conditions.

Cultures that receive dim light attain steady-state growth rates as well as constant pigment contents after 8 to 10 generations. The light dependence of growth, however, is very different from that for pigment synthesis (fig 4). There are clearly 2 responses to light that promote growth; 1, a low energy reaction that saturates at about 0.1 μw/cm^2 and a second, high energy reaction that saturates above 2000 μw/cm^2. At intensities between 0.1 μw/cm^2 and 100 μw/cm^2 the rate of cell increase is virtually independent of light energy. Thus it is noteworthy that carotenoid synthesis is slightly dependent and chlorophyll synthesis strongly dependent on light intensity over the same range.

Action Spectrum. The action spectrum for the low energy reaction was investigated by measuring the steady-state growth rate as a function of wavelength at constant energy. Since cultures must be grown for a minimum of 10 generations before a steady state is established, it was not practical to determine the limiting intensity range separately for each wavelength. For this reason we initially experienced difficulty in obtaining an action spectrum with good definition of the peaks. Intensities of 10 to 20 μw failed to differentiate the peaks on the 1 hand, and on the other, intensities of less than 0.1 μw at many wavelengths did not produce a steady state. We eventually obtained good resolution of the action peaks using an energy level of 0.15 $\pm$ 0.05 μw/cm^2.

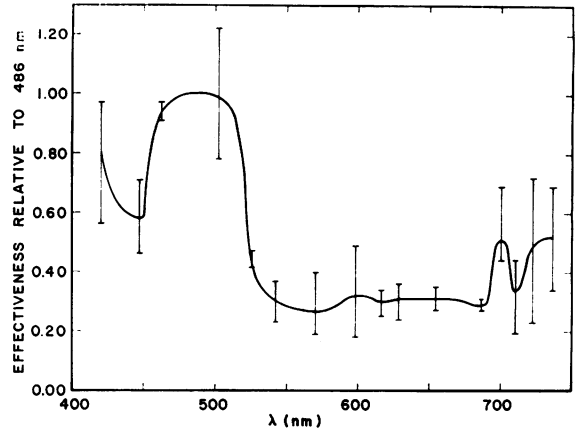

FIG. 5. Action spectrum for the low energy growth response of y-2 as determined with continuous monochromatic irradiations of 0.15$\pm$0.05 μw/cm^2. The data were taken from steady-state cultures that had been under the specified conditions for a minimum of 12 generations. The growth constant at each wavelength relative to that at 486 nm was the measure of effectiveness used. The curve passes through the mean values of 3 to 6 independent determinations at each wavelength. The vertical bars indicate the entire range of experimental values.

Since y-2 cells are capable of the equivalent of 9 to 10 generations in darkness, one can discern very little influence of low energy radiation up to this point. An action spectrum based on the rate of cell increase between the eighth and twelfth generations under low energy irradiation gives only a suggestion of peaks. Irrespective of wavelength, the growth constants at this stage were all between 0.4 and 0.5, which is only slightly below the saturated level for the low energy reaction. By carrying the experiments beyond 12 generations, an action spectrum with good resolution was obtained (fig 5). For reasons that will be partly clarified later, there are at least 3 peaks of effectiveness: at 480 to 502 nm, 700 nm and 736 nm, the latter 2 being considerably lower than the former. In many repeats of these experiments we invariably found that 722 nm and 736 nm were substantially more effective than 710 nm, though the considerable variability in the results with these wavelengths leaves a large measure of doubt about the real form of the action spectrum in the far-red region.

Selection for Other Cell Strains under Conditions of Light-limited Growth. While investigating the action spectrum of steady-state growth we found that a selection for chlorophyll-containing cells took place at certain wavelengths when cultures were

propagated for many generations in rate-limiting monochromatic light. A certain number of mutated or reverted cells would normally be expected in the large populations ($>10^8$ cells) that we used routinely in these experiments. The presence of a new cell line in a culture was readily detected as an increase in chlorophyll content. Positive selection for chlorophyll-containing cells was highly wavelength dependent and took place most rapidly in cultures that received red light. Under these conditions the chlorophyll content of the cultures began to rise abruptly after 15 to 18 generations, or after 5 to 8 generations of selection, if we allow that light does not limit growth for the first 10 generations.

An experiment which illustrates the wavelength dependence of positive selection for green cells is shown in figure 6. Green, light-grown y-2 cells were irradiated with 0.15 ± 0.05 μw/cm^2 at 502 and 700 nm. The rate of cell increase is expressed relative to that in the culture which received 502 nm. For the first 11 days (corresponding to about 13 generations) the rate of increase in the 2 cultures was approximately equal. Light then became strongly limiting at 700 nm relative to 502 nm. The chlorophyll content of these cultures initially decreased to a very low and nearly equal level in accordance with the slow pigment synthesis by y-2 at 0.15 ± 0.05 μw/cm^2. During the interval between the eleventh and nineteenth days of the experiment a dramatic increase in chlorophyll content took place in the culture that received radiation at 700 nm. Over the following 3 days there was little further change in the pigment content of these cells, while their division rate increased more than 2-fold to a level practically equal to that in the 502 nm control. On the twenty-third day the culture which had up to then received 700 nm was irradiated at 486 nm instead. At the same time a portion of the culture which was being irradiated at 502 nm was inoculated into fresh medium and given radiation at 669 nm.

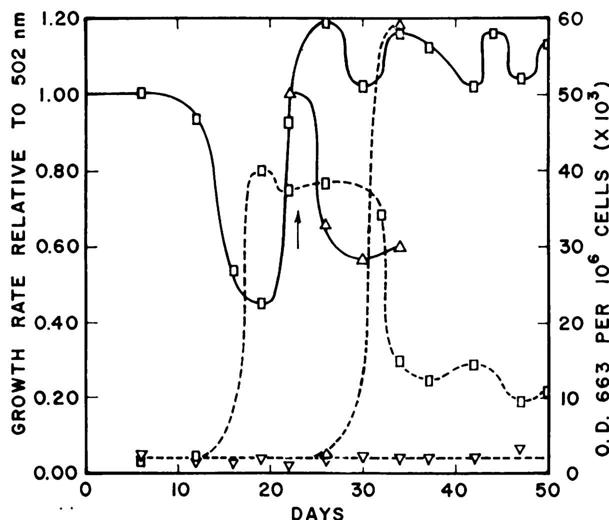

FIG. 6. Takeover of y-2 cultures by a green-in-the dark strain. ————, growth rate relative to 502 nm; - - - - -, chlorophyll content. □'s indicate data from a culture that was irradiated initially with 700 nm and then on day 23 was given 486 nm. △'s represent a moiety of the 502 nm control that was given 669 nm on the twenty-third day. ▽'s signify optical density data for the 502 nm control. The arrow marks the twenty-third day. Continuous irradiation of 0.15 ± 0.05 μw/cm^2 at all wavelengths.

In both instances the effects of altering the wavelengths to which the cultures were exposed were qualitatively in accord with what would be predicted on the basis of the action spectrum for growth and the finding that red light causes selection for green cells while blue light does not. The culture that received 486 nm, having been composed mainly of green cells as a result of selection at 700 nm, returned rapidly to a lower chlorophyll content while it continued to grow even more rapidly than the 502 nm control. After 9 days a new steady-state chlorophyll content was established at a level about one-fourth of the previous one but still 10 times greater than the 502 nm control. We cannot offer a definite explanation of this result, though a likely possibility is that the decrease in chlorophyll content at 486 nm was not accompanied by a return of the y-2 mutant. In contrast with the above, the growth rate of cells transferred from 502 nm to 669 nm quickly declined. A sharp rise in the chlorophyll content of this culture after 8 days (8.0 generations) indicated a rapid takeover by green cells.

Positive selection for green cells, as indicated by an increasing chlorophyll content, inevitably resulted in a restoration of the cell division rate to a level at least equal to that of the unaltered 502 nm control. Typical data are presented in figure 7. The first noticeable acceleration of the growth rate always lagged the appearance of green cells by several generations. Such kinetics result from the fact that small numbers of green cells do not contribute significantly to the overall growth rates of large populations but contain enough chlorophyll to be readily detected in the presence of y-2. For this reason it was an easy matter to eliminate changes in growth rate due to selection as a source of error in the action spectrum by excluding all data from cultures that had begun to turn green.

Given a sufficient number of generations of positive selection, green cells eventually take over light-limited cultures at any wavelength at which absorption by chlorophyll exceeds the absorption by carotenoids (figs 8 and 9). Thus, after roughly 5 to 8 generations of selection in limiting light, only those cultures which were irradiated at 402, 462, 486, and 502 nm had failed to show significant increases in chlorophyll. Figure 9 should not be taken at face value as an action spectrum for the strength of positive selection for wild-type cells, since we do not accurately know the wavelength dependence of chlorophyll synthesis in either the

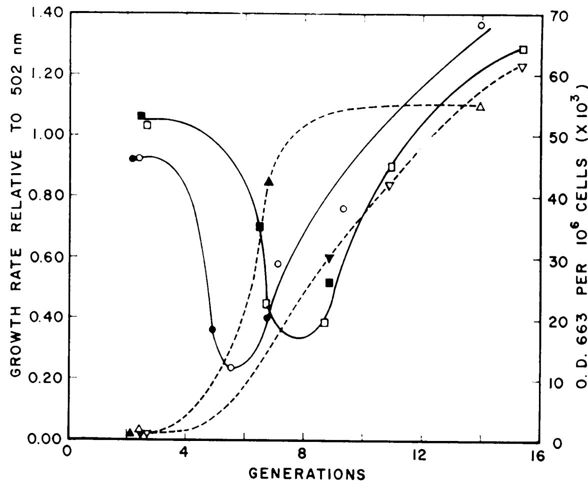

FIG. 7. Changes in the growth rate relative to 502 nm control and in the chlorophyll content of y-2 cultures. The abscissa commences just prior to the onset of light limitation and therefore a number of generations following the start of the experiment. ●,○ relative growth and ▲,△ chlorophyll content of cultures irradiated at 628 nm. ■,□ relative growth and ▼,▽ chlorophyll content of cultures irradiated at 700 nm. Solid and enclosed points represent data from separate experiments. Continuous irradiation of 0.15±0.05 μw/cm² at both wavelengths.

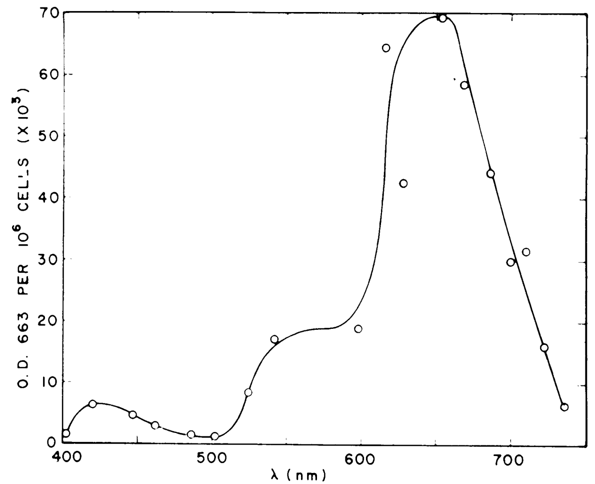

FIG. 9. The chlorophyll content of y-2 cultures after 9 to 11 days of light-limited growth as a function of wavelength. The points were taken from the data shown in figure 8 and from the results of other similar experiments. Continuous irradiation of 0.15±0.05 μw/cm² at all wavelengths.

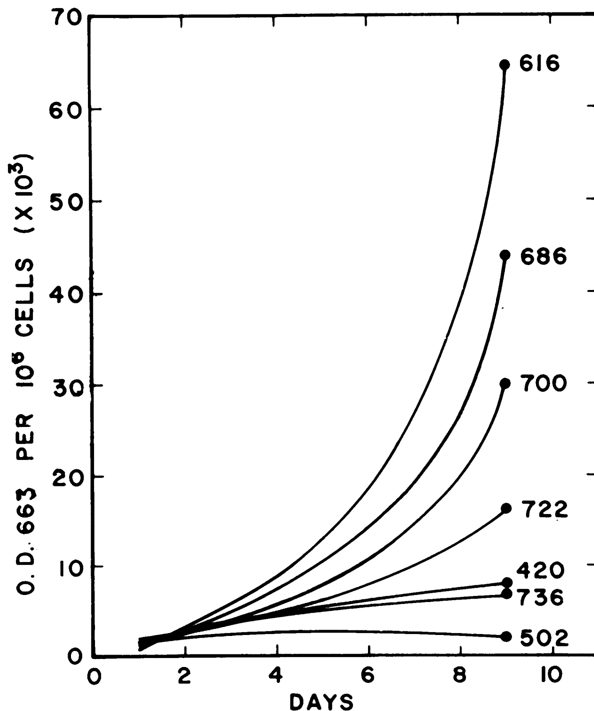

FIG. 8. Kinetics of the chlorophyll contents of light-limited y-2 cultures. Numbers at the ends of the curves refer to wavelengths in nm. The abscissa commences at a time just prior to the earliest rise in chlorophyll content. Continuous irradiation of 0.15±0.05 μw/cm² at all wavelengths.

y-2 or green strains. On the other hand, the results indicate clearly, in a qualitative fashion, the wavelengths at which cells with a relatively high chlorophyll content have a selective advantage over those with a much lower chlorophyll content under conditions where light absorption is limiting growth. This advantage is greatest in the red and orange portions of the spectrum, somewhat less in the yellow and green regions and very slight at 420, 447, 722, and 736 nm. The obvious implication of these results is that both chlorophyll and carotenoids are active as photoreceptors in the special low-energy effect of light on cell division, at least in *C. reinhardi*. The effectiveness of the 2 classes of pigments appears simply to be in proportion to their relative absorbancies at any given wavelength. As appealing as this view may be, we must grant that it is not yet a proven conclusion and that it presents certain difficulties of interpretation which we will discuss in the following section.

Discussion

The majority of green cells accommodate to decreased light intensity by an adaptive increase in chlorophyll content. This behavior is characteristic of such diverse organisms as *Chlorella vulgaris* (13), *Euglena gracilis* (14) and *Acetabularia* (16). The former 2 of these organisms, when grown on a carbon source in very dim light or darkness, contain considerably less chlorophyll than controls grown at photosynthetic intensities. Thus, the chlorophyll content of such facultative mixotrophs attains a maximum at some fairly low light intensity, which in the case of *E. gracilis* is around 90 ft-c (14). In contrast, the chlorophyll content of y-2

is a linear function of light intensity over a 10^5-fold range on a double logarithmic plot. Similar measurements on the wild type strain would now be of interest, since y-2 appears to possess a new type of control of chlorophyll synthesis by light. The log-linear relationship indicates furthermore that the rate of chlorophyll synthesis in y-2 is not simply dependent on the amount of light absorbed by chlorophyll in the cells.

Like many other photosynthetic cells that are capable of growth in the dark, y-2 contains both a light-dependent and a light-independent fraction of carotenoids. The latter is approximately 10 % of the carotenoid complement of light-grown cells. By using steady-state cultures we found that most of the light-dependent carotenoid synthesis takes place at moderate intensities at which an appreciable rate of photosynthesis could be expected. Krinsky and Levine (11) identified 9 carotenoids in extracts of light-grown y-2 cells and found all but 1 of these (zeaxanthin) in 96-hour dark cultures. Thus, the effect of light may be largely quantitative.

Evidence from the 2 organisms in which the special light requirement for organotrophic growth has been studied indicates that the critical product of the light reaction is present in great excess in light-grown cells. This can be inferred from the fact that both *Chlorella vulgaris* and y-2 are capable of divisions when first placed in darkness but that growth eventually ceases after the equivalent of about 5 (9) and 9 generations, respectively. Evidently the abilities to assimilate substrate and to synthesize principal cellular constituents are not the limiting factors in dark growth. Moreover, cells that have ceased to divide in the dark are considerably larger than growing cells and conspicuously packed with starch grains (10; 5; 7). This slight evidence invites the opinion that the dark block may specifically involve some step in the developmental cycle, in particular one that precedes the formation of daughter cells.

The action spectrum of Karlander and Krauss (9) for a low-energy stimulation of division in *Chlorella vulgaris* contains peaks at 425 and 575 nm. These wavelengths lie in the troughs of the y-2 action spectrum. This discrepancy must be resolved if one is not to conclude that the reactions in the 2 organisms are basically different. A probable source of disagreement comes from the fact that the pigment content of the strain of *C. vulgaris* used by Karlander and Krauss (8) was highly sensitive to wavelength at an intensity of 0.2 $\mu w/cm^2$, the same as that used in investigating the action spectrum. In contrast, the pigment content of y-2 at that intensity is much lower and shows relatively little dependence on wavelength. For these reasons the y-2 action spectrum is probably more realistic.

The energetics of the special light effect have little in common with photosynthesis and yet there is fairly strong evidence, in the case of y-2 at least, that photosynthetic pigments are among the active photoreceptors. Saturation of the special effect occurs at about 0.1 $\mu w/cm^2$ which is less than 1 % of the intensity needed to compensate respiration in most green cells. Moreover, the optical density of y-2 cells produced at such intensities is only a small fraction of that of light-grown cells, especially at the red end of the spectrum. On the basis of these considerations alone, it seems doubtful that light-limited steady-state cultures of y-2 would photosynthesize at more than one-thousandth of the compensation rate of phototrophic cells.

Further evidence that argues against the participation of photosynthesis in the low energy effect has been obtained with 96-hour dark-grown cultures of y-2. As mentioned earlier, such cells respond by dividing after irradiations with monochromatic light and so are physiologically competent *vis-a-vis* the low energy reaction. On the other hand, 96-hour dark cells do not evolve detectable quantities of oxygen when illuminated with bright light (5), have only a disorganized trace of lamellar structure (6), and possess a higher proportion of chlorophyll b than do light-grown cells (12). The latter observation stands in contrast with our action spectrum for the low energy response in which the effectiveness of wavelengths absorbed by chlorophyll b is relatively poor.

When 96-hour dark cultures are placed in bright light, chloroplast development ensues rapidly after a short lag. The appearance of organized lamellar structure and the onset of oxygen evolution are coincident after 3 hours of exposure to light (6). At this stage in regreening the cultures contain about 0.5 μg chlorophyll/10^6 cells, or roughly 50 times as much as steady-state cultures that have received 0.08 $\mu w/cm^2$ of white light. A further apparent contrast between the low-energy effect and photosynthesis is to be found in the virtual independence of steady-state cultures of light intensity within the range of 0.1 to 100 $\mu w/cm^2$. Since chlorophyll synthesis increases regularly with light intensity throughout this range, cells grown at the higher intensity will absorb more light than those grown at the lower intensity by a factor of several orders of magnitude. Thus, an increased absorption by chlorophyll per se does not contribute to growth so long as the intensity remains below the range in which respiration is compensated in most green plants. This suggests that the sharp rise in growth rate with increasing intensity above 100 $\mu w/cm^2$ is probably attributable to net photosynthesis.

The arguments against an interpretation of the low energy effect based on photosynthesis are opposed by 3 lines of evidence that suggest rather compellingly that photosynthetic pigments are involved as photoreceptors. A) The action spectrum shows peaks of effectiveness between 462 and 502 nm, a region in which carotenoids absorb strongly, and between 700 and 736 nm, where the long wavelength form of chlorophyll a is known to absorb (17). Though our results indicate 2 separate peaks

of activity in the latter region at 700 nm and 722 to 736 nm, it is possible that this is an artifact due to random scatter of the data and that there is only a single response band in the near IR. B) When selection for a green cell type took place in growth-limiting monochromatic light, we found a consistent correlation between an increasing chlorophyll content and an accelerating growth rate. It is possible that the green cells were favored in these experiments by virtue of some quality other than their higher chlorophyll content, but this interpretation is countered by the fact that the 2 strains probably differ by only a single gene that controls the chlorophyll content at very low light intensities. Corroborative results have recently been reported by Hudock and Bart (4) who found that green, wild-type cells took over in y-2 cultures that were kept in darkness for a week or more. C) The wavelengths at which selection favored the green strain over y-2 were precisely those at which chlorophyll would be expected to have a greater absorption than carotenoid in a green cell, namely at 420 to 447 nm, and at all wavelengths longer than 502 nm. The yellow mutant cells were not at a disadvantage at 402 nm and within the range of 462 nm to 502 nm, wavelengths at which chlorophyll absorption would be relatively slight.

All 3 of the above results lead independently to the conclusion that at least some forms of chlorophyll act as photoreceptors in the low energy effect on cell increase. Moreover, results A and C in addition implicate carotenoids in the appropriate wavelength regions. How then can one reconcile the arguments presented earlier that seemed inconsistent with the participation of photosynthesis in the low energy effect? Any answer to this question must for the present be considered highly speculative. One possibility that is consistent with the results obtained so far is that in y-2 the low energy effect is mediated by photosystem I only. The growth limiting factor would then be a product of system I that is required in small amounts for some purpose other than the reduction of carbon dioxide. This interpretation would account for the absence of an action peak in the chlorophyll b region, even though chlorophyll b is known to be present (12). Furthermore, it is necessary to postulate that in y-2 only carotenoids and the long wavelength form of chlorophyll a are effective but that in green cells the shorter wavelength forms of chlorophyll can also function as photoreceptors. In support of this postulate is the finding that selection favoring the wild type over y-2 is very high at wavelengths absorbed by the short wavelength forms of chlorophyll but is weak above 700 nm. This interpretation of the low energy effect offers opportunities for experimental testing.

Literature Cited

1. Bruinsma, J. 1961. A comment on the spectrophotometric determination of chlorophyll. Biochim. Biophys. Acta 52: 576–78.
2. Danforth, W. F. 1962. Substrate assimilation and heterotrophy. In: Physiology and Biochemistry of Algae. R. A. Lewin, ed. Academic Press, New York. p 99–123.
3. Hellebust, J. A. and J. Terborgh. 1967. Effects of environmental conditions on the rate of photosynthesis and the activities of ribulose-1,5-diphosphate carboxylase and aldolase in *Dunaliella tertiolecta* Butcher. Limnol. Oceanog. In press.
4. Hudock, G. A. and C. Bart. 1967. Responses of a mutant strain of *Chlamydomonas reinhardi* to prolonged organotrophic growth. Plant Physiol. 42: 186–90.
5. Hudock, G. A. and R. P. Levine. 1964. Regulation of photosynthesis in *Chlamydomonas reinhardi*. Plant Physiol. 39: 889–97.
6. Hudock, G. A., G. C. McLeod, J. Moravkova-Kiely, and R. P. Levine. 1964. The relation of oxygen evolution to chlorophyll and protein synthesis in a mutant strain of *Chlamydomonas reinhardi*. Plant Physiol. 39: 898–903.
7. Griffiths, D. J. 1965. The accumulation of carbohydrate in *Chlorella vulgaris* under heterotrophic conditions. Ann. Botany London 29: 347–57.
8. Karlander, E. P. and R. W. Krauss. 1966. Responses of heterotrophic cultures of *Chlorella vulgaris* Beyerinck to darkness and light. I. Pigment and pH changes. Plant Physiol. 41: 1–6.
9. Karlander, E. P. and R. W. Krauss. 1966. Responses of heterotrophic cultures of *Chlorella vulgaris* Beyerinck to darkness and light. II. Action spectrum for and mechanism of the light requirement for heterotrophic growth. Plant Physiol. 41: 7–14.
10. Killam, A. and J. Myers. 1956. A special effect of light on the growth of *Chlorella vulgaris*. Am. J. Botany 43: 569–72.
11. Krinsky, N. I. and R. P. Levine. 1964. Carotenoids of wild type and mutant strains of the green alga, *Chlamydomonas reinhardi*. Plant Physiol. 39: 680–87.
12. McLeod, G. C., G. A. Hudock, and R. P. Levine. 1963. The relation between pigment concentration and photosynthetic capacity in a mutant of *Chlamydomonas reinhardi*. In: Photosynthetic Mechanisms in Green Plants. Natl. Acad. Sci. Natl. Res. Council Publ. 1145: p 400–08.
13. Steeman Nielsen, E., V. Kr. Hansen, and E. Jorgensen. 1963. The adaptation to different light intensities in *Chlorella vulgaris* and the time dependence on transfer to a new intensity. Physiol. Plantarum 15: 505–17.
14. Stern, A. I., H. T. Epstein, and J. A. Schiff. 1964. Studies of chloroplast development in *Euglena*. VI. Light intensity as a controlling factor in development. Plant Physiol. 39: 226–31.
15. Sueoka, N. 1960. Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardi*. Proc. Natl. Acad. Sci U. S. 46: 83–91.
16. Terborgh, J. and K. V. Thimann. 1964. Interactions between daylength and light intensity in the growth and chlorophyll content of *Acetabularia crenulata*. Planta 63: 83–98.
17. Vidaver, W. 1966. Separate action spectra for the two photochemical systems of photosynthesis. Plant Physiol. 41: 87–89.