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THE ACTION SPECTRUM OF LIGHT INDUCED AGGREGATION IN *POLYSPHONDYLIUM PALLIDUM*, AND A PROPOSED GENERAL MECHANISM FOR LIGHT RESPONSE IN THE CELLULAR SLIME MOLDS

WARREN R. JONES, III AND DAVID FRANCIS

Department of Biological Sciences, University of Delaware, Newark, Delaware 19711

For a long time it has been known that light has a stimulatory effect of aggregation in the cellular slime molds. The response has been recorded variously by different authors as an effect on the time of aggregation, the number of aggregates, or the size of the aggregates, and it has not been clear whether there is a single primary response common to these differing effects. The picture is further confused by the fact that the details of the response are different from one species to another.

The work that we report here is intended to carry one step further the understanding of the light response in one species, *Polysphondylium pallidum*, by providing an action spectrum for the response. In addition, we propose a general mechanism which may be responsible for the various forms of the light response.

Materials and Methods

We used *Polysphondylium pallidum*, strain WS 320, kindly supplied by K. B. Raper. Stock cultures were kept on *Escherichia coli*, strain 281, on agar made with 0.2% Cerophyll. (Cerophyll consists of powdered cereal grass leaves and is available from Cerophyll Laboratories, Kansas City, Missouri.) Amebae for experiments were grown axenically by inoculating spores from the stock plates into the following medium, modified from Sussman (1963):

Lecithin (Eastman Kodak, practical grade)	−0.4 g
Proteose peptone (Difco)	−10 g
Glucose	−1 g
$Na_2HPO_4 \cdot 7H_2O$	−0.72 g
KH_2PO_4	−1.45 g
Streptomycin sulfate	−10 mg
Dist H_2O	−1 liter

The flasks were incubated on a shaker at 23–26° C, in constant fluorescent light of ~50 foot candles intensity. After 3–4 days the amebae were harvested by centrifugation, washed once in cold Bonner's salt solution (Bonner, 1947) and dispensed as 10 λ drops onto plates of 1.5% Difco purified agar made with distilled water, which had been poured 2–3 days in advance. This procedure of plating was initiated by Konijn and Raper (1961). The drop soon dries, leaving a population

of amebae which remain within a small area on the agar surface. After plating the dishes were incubated in constant darkness at 21 ± 1° C.

At a time and in a manner to be detailed in the results section, the plates were exposed to light for a brief period then returned to darkness.

As light sources we used either a General Electric cool white fluorescent lamp at 4.1×10^3 ergs/cm^2/sec intensity, or monochromatic light provided by a microscope lamp in combination with Schott interference line filters. Approximately 99% of the energy transmitted is within a 20 millimicron interval with these filters. The brightness of the lamp was controlled by a Variac transformer, and a four-inch water bath was interposed in the light beam to absorb infrared energy. The intensity was measured with a YS1-Kettering model 65 radiometer and a Photovolt Corporation model 520M photometer. The photometer was necessary for measuring the lower intensities and was calibrated (in terms of ergs/cm^2/sec) at each wavelength by using the radiometer.

Six hours after light exposure the plates were taken from the incubator and the number of aggregation centers per drop was recorded.

TABLE I

Effect of length and time of exposure on density of aggregation

	Length of exposure (min)			
	0 (Background)	1/4	2	30
Time at which exposed (Hours after plating)				
1	26 centers/drop	26	26	32
5	24	33	46	43
17–20	32	43	40	44

RESULTS

Response to brief periods of illumination

Our first experiment was designed to select an appropriate time and length of exposure for later use in determining the action spectrum.

The amebae were plated and incubated as described above, then exposed to fluorescent light for varying brief periods, then returned to the dark and later the aggregates counted. The results of one typical experiment are shown in Table I and may be summarized as follows:

(a) It is clear that the background number of aggregates is rather high: 24–32 centers per drop appear without any light exposure. This is in contrast to Kahn's (1964) results with a similar experiment where the background was near zero. The background appears to increase slightly from 5 to 17–20 hours, and repeated tests show that this is generally true (Fig. 1).

(b) In the two cases where the number of aggregates in exposed plates is clearly above background, *i.e.*, at 5 and 17–20 hours, a two-minute exposure is as effective in inducing aggregates as a 30-minute exposure. Kahn (1964) found a similar result.

(c) The time at which exposure is most effective in increasing the number of aggregates is at 5 hours after plating. The total number of aggregates per drop is essentially the same at 17 hours, but the background is higher in that case. Exposure at one hour after plating has almost no effect. This result is in apparent conflict with Kahn's (1964) conclusion that the sensitivity to induction decreased from 1 hour to 6 hours. A possible cause of the disagreement is that Kahn used amebae grown in darkness, whereas ours were light grown; for it seems reasonable that amebae which have been taken from constant light should be insensitive to a brief flash given only one hour later. Also, growth in light might cause induction of some center forming cells before plating, and so might explain our high background.

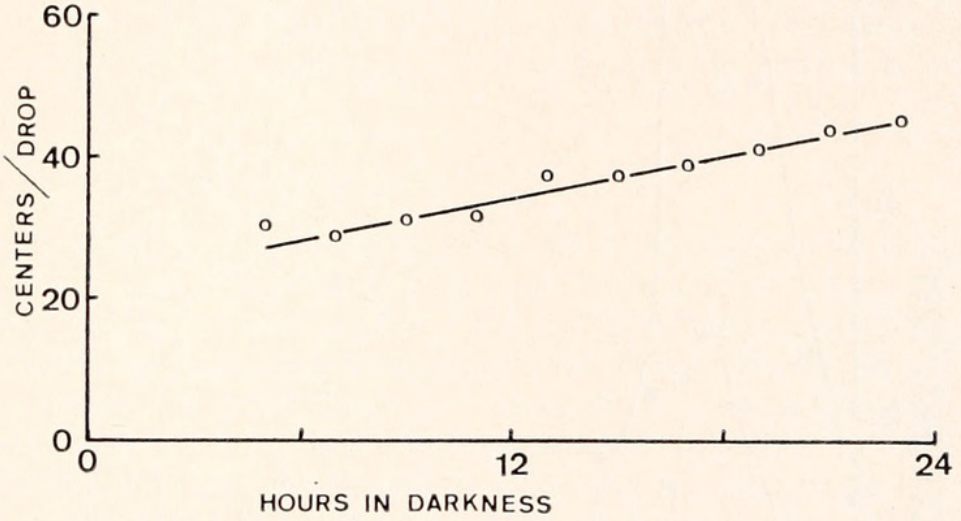

FIGURE 1. Increase in number of aggregations in cell populations kept in constant darkness. The drops were plated at T = 0. Points are averages of counts made on 6 drops. Culture harvested at 4.0×10^4 cells/ml, plated at 5.0×10^5 cells/ml.

Action spectrum

We concluded that 2 minutes of light given at 5 hours after plating would be a suitable exposure to use in determining an action spectrum and that intensities around 4×10^3 ergs/cm^2/sec would be relevant. The experiments were performed in the same way as above, except that the amebae were exposed to monochromatic light instead of white light. The same intensity was used at each wavelength, so as to give an *equal energy* action spectrum. The results of three separate experiments at two intensities are shown in Figure 2. There are two main peaks of activity, one at 475 mμ, one at 675 mμ.

DISCUSSION

The many workers who have studied the light response of cellular slime molds have recorded effects on a variety of processes. The more important of these are the following:

(*a*) *Effect on the time of aggregation*

Most experimental arrangements have used cells plated on agar in the absence of food. After plating a certain time elapses before aggregation begins, and this

time is one phenomenon which is influenced by light. Konijn and Raper (1965) found that aggregation occurs soonest in constant light in *P. pallidum,* which in this respect differs from *D. discoideum* and *A. rosea* where a dawn following a night of certain length is optimal (Konijn and Raper, 1965; Reinhardt, 1968). In all of these cases, light has its effect on post-vegetative, preaggregative cells,

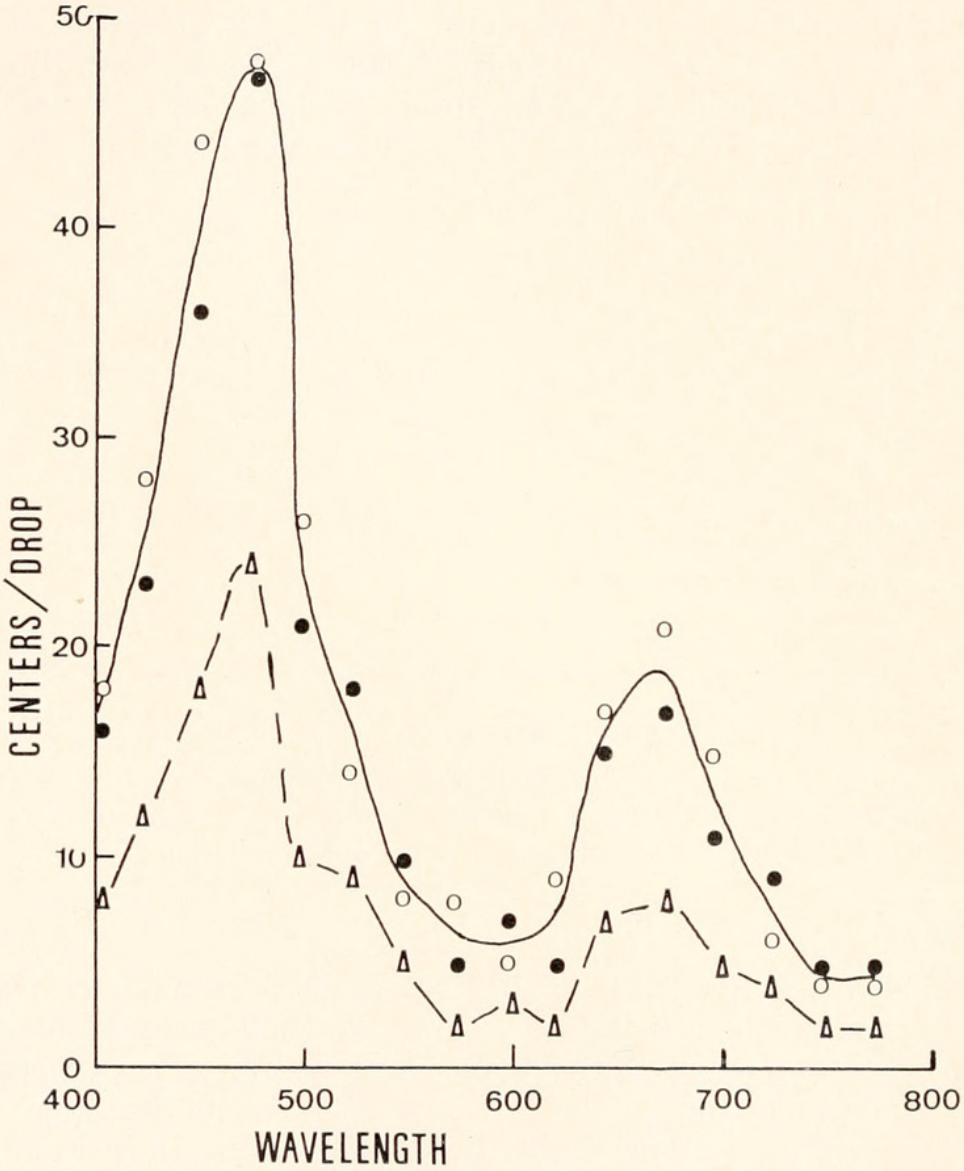

FIGURE 2. The action spectrum of light induced aggregation. Centers/drop above background (ordinate) are plotted against wavelength in mμ (abscissa). "Background" is the number of centers which appear in drops kept in constant darkness. Each point is the average of counts made on 6 drops. ○, ● indicate results of two separate experiments using 9.2×10^3 ergs/cm^2/sec at each wavelength. Cultures were harvested at 1.2×10^6 cells/ml, and plated at 5×10^5 cells/ml; background equals 45 and 50 centers/drop. △ indicates results of a third experiment using 4.8×10^3 ergs/cm^2/sec at each wavelength. Cultures were harvested at 1.5×10^6 cells/ml, and plated at 1.0×10^6 cells/ml; background equals 48 centers/drop.

and must induce in them some cellular process which leads to aggregation. Some observations by Shaffer (1961) give a hint as to what this process might be. He found that aggregations in *P. violaceum* are started by special founder cells which apparently secrete large amounts of acrasin, for they directly attract neighboring cells. Furthermore, Shaffer noted that the number of founders increased sud-

denly following a flash of light. This suggests that light can induce the production of acrasin in at least some cells. Founder cells also occur in *P. pallidum* (Francis, 1965), and quite probably their appearance is stimulated by light in this species as well, although no direct observations on this point have been made.

(*b*) *Effect on the number of centers per unit area*

In all species which have been studied the number of aggregations per unit area is higher when the population has been exposed to light (*D. discoideum*—Konijn and Raper, 1966; *P. pallidum*—Kahn, 1964; *A. rosea*—Reinhardt and Mancinelli, 1968). Where light flashes have been employed to investigate this phenomenon it is clear that one sensitive period is the same preaggregative phase mentioned above which is several hours before macroscopic aggregations appear. Perhaps this means that the primary event here is also the induction of founder cells.

(*c*) *Effect on the size of aggregates*

This measure is partly correlated with the effect on number of centers, since if there are more centers per unit area (and if all of the amebae enter some center) each center must necessarily be smaller. Especially when this criterion of light stimulation is used it becomes clear that light has an effect not only on preaggregative cells, but also on the later stages of aggregation. For example, Reinhardt (1968) has noted that in *A. rosea,* light causes the breaking up of large old aggregates. Because of this second period of sensitivity to light it is important to note the time after initial exposure to continuous light at which aggregates are counted. The number of induced aggregates may be quite different when counted at 6 hours after initial exposure when aggregates are just becoming macroscopically visible, and when counted at 24 hours when the first formed aggregates have possibly been dissolved and new ones have formed. So as to make doubly sure of avoiding these secondary effects we counted aggregates at 6 hours, even though in our experiments the developing aggregates were kept in darkness after the brief exposure.

(*d*) *Effect on orientation of the pseudoplasmodium*

In several species the migrating or stalk-forming pseudoplasmodium orients toward a source of light. The effect occurs at the tip of the body and on the side away from the light source, and may possibly be an increase in the motive power of the amebae in this zone (Francis, 1964).

In view of such variety in the expression of response to light and in the diversity of the behavior of different species, one may wonder if there can in fact be a common mechanism of reaction. Nevertheless we want to put forward a possible basic mechanism, which is suggested by an analogy of the phenomena of aggregation with the processes of light reception in the metazoan eye. As summarized by Bitensky, Gorman and Miller (1971), the response in the vertebrate retina consists of light trapping by the pigment 11-cis retinal, followed by hyperpolarization of the retinal cell membrane. In invertebrate eyes the linkage is in the opposite direction and light causes *de*polarization (Miller, Gorman and Bitensky,

1971). Bitensky showed that the intermediate process between light reception and hyper- or depolarization involves a change in activity of adenyl cyclase. It would seem that this is followed by a corresponding change in concentration of cyclic-AMP, which then in some way alters the permeability of the membrane to Na^+, resulting in the change in membrane potential.

These events may be compared with what happens during aggregation in the cellular slime mold. Here Bonner's group (Bonner, Barkley, Hall, Konijn, Mason, Keefe and Wolfe, 1969) has recently shown that a key event is the sudden increase in cyclic-AMP, at least in *D. discoideum*. Cyclic-AMP is one naturally occurring acrasin, and attracts cells to the forming centers. How the chemotaxis operates is not yet completely understood, but it is known that cyclic-Amp can change the membrane permeability to Ca^{++} (but not Na^+) in the cells of *D. discoideum* (Chi and Francis, 1971), and that Ca^{++} activates the ATP-ase activity of contractile proteins in amebae of cellular slime molds (Woolley, 1970). These known similarities between the retina and aggregation suggest that the two systems might

TABLE II

Proposed parallels between the processes of aggregation and vision occurring after light stimulation

	Retina	Aggregation
1	Light + 11-cis retinal	Light + unknown pigment
2	Change in C AMP production by adenyl cyclase activation, or inactivation	Changed production and release of C AMP or other acrasin by attracting cells
3	Change in Na^+ permeability and membrane potential of retinal cell	Local change in Ca^{++} permeability and intracellular Ca^{++} in responding cells
4	Impulse in optic nerve	Chemotaxis of responding cell, caused by assymetric operation of contractile system

be similar in other aspects as well. In particular, it seems useful to hypothesize that the unique immediate effect of light on the cellular slime molds is to induce production of cyclic-AMP or acrasin. The suggested analogy is outlined in Table II.

Some of the light stimulated reactions of cellular slime molds already listed are explicable by an increased acrasin production in some or all cells. The increase of visible founder cells following light exposure is one of these, since the easiest interpretation of a founder cell is that it is a cell secreting exceptional quantities of acrasin. Early or more frequent induction of founder cells will lead to faster and more numerous aggregates, as mentioned earlier, and explain the light stimulation of aggregation. The breaking up of already formed centers is not so obviously derived from increased acrasin production, although it is conceivable that the chemotactic gradient surrounding an aggregate might be disrupted if many of the peripheral cells suddenly begin to produce large amounts of acrasin. This could explain the case of *A. rosea*. Further, Konijn (personal communication) has noted that a very high concentration of C AMP in the agar substrate brings about disintegration of previously existing centers. Phototaxis is a phe-

nomenon which might easily be caused by a chain reaction leading from light stimulation through acrasin production to altered cell movement, as outlined above. It should be carefully noted that we here used cyclic-AMP and acrasin interchangeably. In actual fact, of course, cyclic-AMP is the acrasin for only some of the *Dictyostelium* species but not for *Polysphondylium* (Bonner *et al.*

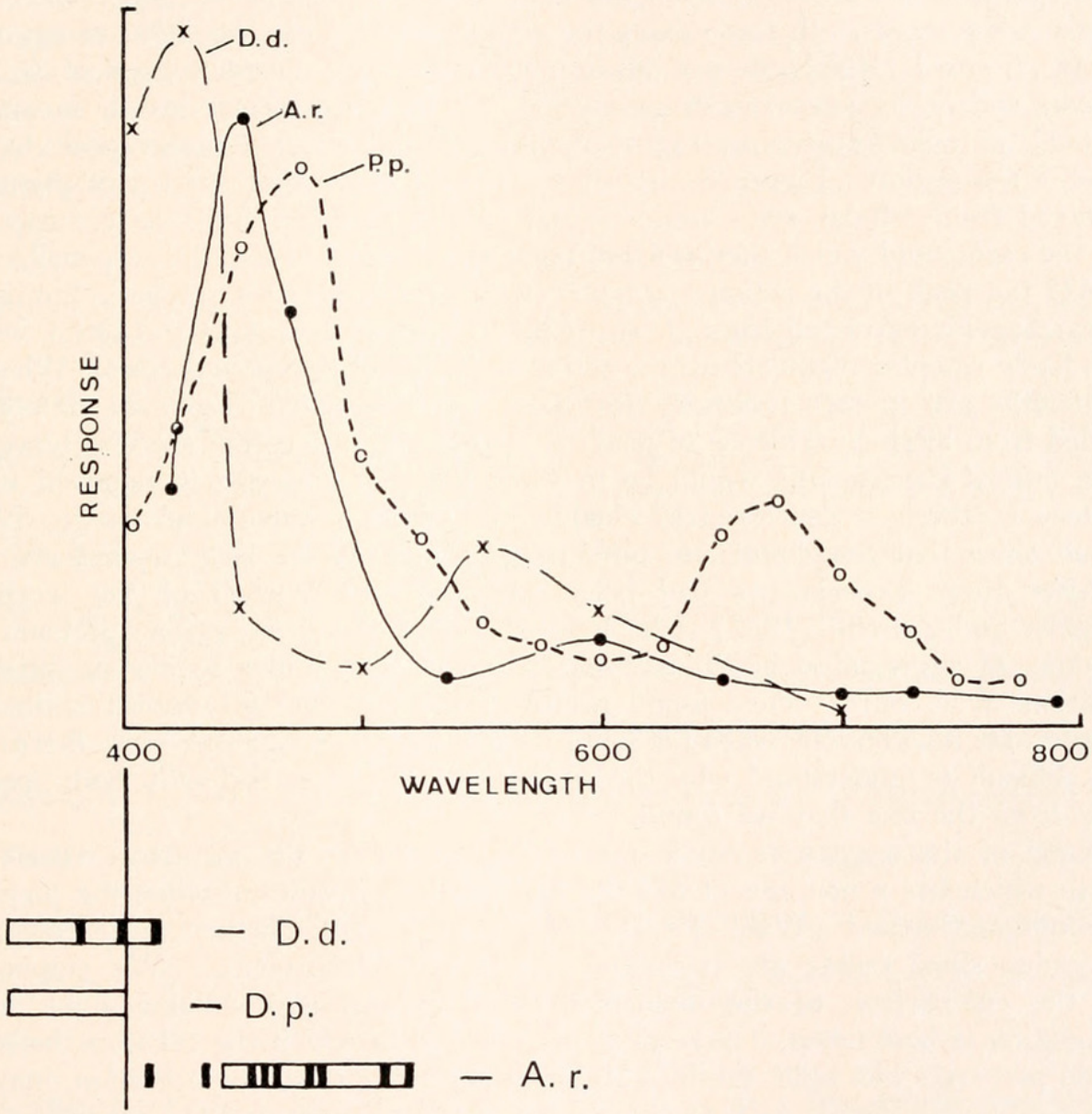

FIGURE 3. Comparison of action spectra (above) and absorption spectra (below) for three different species. For action spectra the units of response are different in each case. D.d. indicates phototaxis of *D. discoideum* and *D. purpureum* (Francis, 1964). The response is the reciprocal of the amount of energy in ergs/cm^2/sec necessary to produce a standard phototactic turn. A.r. indicates induction of aggregation in *A. rosea* (Reinhardt and Mancinelli, 1968). The response is the number of centers per standard drop of amebae. Equal intensities were not used at all wavelengths, but the intensity at 450 mμ was weaker than the rest so that this peak is real. P.p. indicates induction of aggregation in *P. pallidum*, from Figure 2. For absorption spectra the boxes indicate the regions of absorption, bars indicate peaks in absorption. Abbreviations are D.d, ethanol extract of carotenoids of *D. discoideum* (Staples and Gregg, 1967); D.p., cell-free liquid from spore heads of *D. purpureum* (Francis, 1964); A.r., hexane solutions of several carotenoids of *A. rosea* (Fuller and Rakatansky, 1966).

1969), and it is an additional assumption that in *Polysphondylium* light has the same effect on production of the unknown acrasin as it is postulated to have on production of cyclic-AMP in *Dictyostelium*. The main value of the hypothesis is that it provides a mirror within which these several phenomena can be viewed from a new angle. We hope that it will be of use in suggesting new experiments.

Action spectra have been determined for light reactions of two other species of cellular slime molds. In these cases the responses were the induction of aggregation in *A. rosea* (Reinhardt and Mancinelli, 1968) and the phototaxis of *D. discoideum* and *D. purpureum* (Francis, 1964). The three spectra are by no means identical, as may be seen in Figure 3, although all show a major peak in the 424–475 mμ region. Figure 3 also shows several absorption spectra of pigments extracted from cellular slime molds. Staples and Gregg (1967) have suggested that the carotenoid which they isolated from *D. discoideum* may be responsible for the 425 mμ peak in the action spectrum of phototaxis for this species. Similarly, the carotenoids extracted from *A. rosea* by Fuller and Rakatansky (1966) would seem likely receptor pigments for the action spectrum of the same species. Whether carotenoids are in fact necessary for these light mediated responses cannot be decided from mere coincidence of peaks in action and absorption spectra, however. More crucial experiments would be to show that the response is removed when carotene synthesis is depressed by chemical inhibitors (Staples and Gregg, 1967) and to show that nonphototactic mutants (Loomis, 1970) lack carotenoids.

After these experiments had been completed we learned of the work of Kientzler and Zetsche (1972), who have also determined an action spectrum for induction of aggregation in *Polysphondylium pallidum,* under somewhat different conditions from ours. They found peaks at 460 and 600 mμ, which shifted to 450 and 550, respectively, when the intensity and length of exposure were increased. It is difficult to understand why the spectral shift occurs, and why both spectra differ from the one that we found.

None of the spectra resemble the action spectra of the metazoan visual response which has a maxium of 375 mμ and another at 500 mμ when the pigment is protein-conjugated (Wald, 1968). Nor is it known whether the carotenoids of cellular slime molds are normally associated with proteins. We emphasize that the comparison of the metazoan visual system and cellular slime mold aggregation is best taken at present as an analogy in which the relations between certain processes like light, cyclic AMP, and permeability to a specific ion, may be similar, although details such as the nature of the pigment and the particular ion to which the membrane becomes permeable are different in the two cases. As yet we have much too little evidence to suggest any true evolutionary homology of the two systems.

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Summary

In the cellular slime mold, *Polysphondylium pallidum,* aggregations are induced by a brief period of light coming after a long (5 hour) dark period. The

action spectrum of this response was determined by illuminating small populations of amebae with a constant sub-saturation amount of light at each of several different wavelengths and measuring the number of aggregates induced in each case. Peaks in response occurred at 475 and 675 mμ. One of these peaks falls on the absorption maximum of hexane extracted carotenoids from another cellular slime mold, *Acrasis rosea,* suggesting that the receptor pigment may be a carotenoid.

The light responses of the cellular slime molds are briefly reviewed. It is suggested that the events during light stimulation of aggregation and during other light induced responses may be similar to those which occur during light stimulation of the metazoan retina. In that case, the initial cellular even common to all light responses of the cellular slime molds may be the stimulation of cyclic-AMP production.

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