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THERMOTACTIC AND PHOTOTACTIC RESPONSES OF

DICTYOSTELIUM DISCOIDEUM PSEUDOPLASMODIA

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THERMOTACTIC AND PHOTOTACTIC RESPONSES OF *DICTYOSTELIUM DISCOIDEUM* PSEUDOPLASMODIA

by

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ABSTRACT

Thermotactic and Phototactic Responses of
Dictyostelium Discoideum Pseudoplasmodia

By

Donna Rae Fontana

Thermotactic and phototactic responses of Dictyostelium discoideum pseudoplasmodia were examined. The thermotactic response of strain AX-2 was dependent on the temperature at which the amoebae grew and developed into pseudoplasmodia. Bulk lipid fluidity, as determined with electron spin resonance spectroscopy, was independent of development temperature and therefore the hypothesis that a change in bulk fluidity is the mechanism for the thermal adaptation of the thermotactic response appears untenable.

Thermotaxis mutants of D. discoideum were characterized and three types of mutants were found. Some mutants exhibited altered positive and negative responses. One mutant, HO428, demonstrated normal positive thermotaxis but lacked negative and another mutant had a normal negative response but reduced positive. These results suggest that, in Dictyostelium discoideum, positive and negative thermotaxis have genetically separable pathways and that these pathways converge at some point(s) during transduction.

When the stimulus-dependence of the thermotactic response was studied with the parental strain, HL50, and

some of the mutants, HL50 and one mutant demonstrated a stimulus-dependent change in the sign of the response. This mutant, HO813, had previously shown a reduced positive thermotactic response and a slightly reduced negative response. The stimulus required to change the sign of the response was greater for HO813 than for HL50. When comparing the gradient midpoint temperatures at which HL50 and HO813 switch from negative to positive thermotaxis, at a constant stimulus strength, with HO813 the switch occurred at a higher temperature. These results, along with those obtained with HO428, strongly support the conclusion that there are two concurrent pathways operating during D. discoideum thermotaxis. One pathway is thought to mediate positive thermotaxis and the other negative. The competition between these pathways would determine the sign of the response.

An examination of the phototactic responses of the thermotactic mutants suggested a relationship between positive thermotaxis and phototaxis. These responses exhibited similar temperature-dependences which implies that the two responses may share steps in their transduction pathways. These studies also indicated that Dictyostelium discoideum senses absolute temperature and not just temperature gradients.

DEDICATION

To my father and mother, Edwin and Dorothy Bernardoni

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INTRODUCTION

Dictyostelium discoideum is a cellular slime mold which is remarkably sensitive to its environment. Because it is a eukaryotic organism, has a life cycle which includes single celled and multicellular stages, and because it can be cultured easily, it has become a model system for the study of sensory transduction. In this dissertation I deal with the thermotactic and phototactic responses of *D. discoideum* pseudoplasmodia.

The dissertation includes a literature review which contains a brief description of the life cycle of *D. discoideum* and a summary of its sensory responses (also see 1). The responses of the slug are discussed first because, with the exception of chemotaxis, these responses were observed before their amoebal counterpart and are better characterized. Chapter 1 is a description of materials and methods which are used throughout the dissertation. Chapters 2 through 5 are written in manuscript form and are similar to manuscripts which have been submitted for publication. In Chapter 2, the proposal that the adaptation of the pseudoplasmodial thermotactic response involves membrane lipids is examined. Chapters 3 and 4 contain a description of the thermotactic responses of the parental

strain, HL50, and mutants selected for aberrant thermotaxis. In Chapter 5, the phototactic responses of the thermotactic mutants are described and a possible relationship between pseudoplasmodial thermotaxis and phototaxis is suggested. The results presented in this dissertation are then summarized and recommendations for future work are given.

REVIEW OF LITERATURE

Life Cycle

The complexity of the Dictyostelium discoideum life cycle has attracted biologists since this species was discovered in 1935. As myxamoebae, D. discoideum grows vegetatively utilizing bacteria as a food source. When the food supply is exhausted, the amoebae enter a period of rapid metabolic change called interphase. After interphase, the amoebae begin to aggregate using cyclic AMP (cAMP) as a vehicle for cell-cell communication. Aggregation results in the formation of discrete mounds containing up to 10^5 amoebae. A tip forms on top of the mound and this tip then extends upward until the mound resembles a finger-like structure. This structure falls over and begins to crawl. This crawling, multicellular mass is called a pseudoplasmodium, grex, or slug. After a period of migration, the apex of the slug ceases migrating and this causes the slug to round up. These apical cells then form a vacuolated stalk and the cells from the rear of the slug form either the basal disk which supports the stalk or they sporulate and the resultant spores reside in the sorus on top of the stalk. This process, which results in the formation of a fruiting body, is called culmination. The spores can

then be dispersed by wind or rain and hopefully will land in a hospitable environment. After dispersal, the spores germinate and produce the myxamoebae which completes the life cycle. For a review of this see references (2,3,4).

Slug Phototaxis

Slug phototaxis was first described by Raper (2). He observed that slugs migrate directly toward a light source with their long axes approximately parallel to the beam and their apices, which receive the stimulus, pointing toward the light. Bonner et al. attempted to measure the sensitivity of the slugs to various wavelengths of light and observed that all wavelengths in the 380 to 700 nm range were capable of eliciting an approximately equal response (5). Francis, by reducing the intensity of the light, was able to generate an action spectrum which had peaks at 425 nm and 550 nm (6). With a different technique, Poff et al. obtained similar results (7). They were also able to detect a light-induced absorption change near 411 nm when D. discoideum amoebae were irradiated with 500 nm light. The relative effectiveness of wavelengths was the same whether phototaxis or this light-induced absorption change was used as a response (7,8). Utilizing this light-induced absorption change as an assay, Poff et al. were able to purify, 2000-fold, the pigment responsible for the change and probably responsible for slug phototaxis (9). The absorption spectrum of the pigment showed a strong Soret band with a maximum at 430 nm and weak absorption in the 520

to 600 nm region. This pigment, phototaxin, was an iron-containing, high-spin, heme protein with a molecular weight of 240,000 (8,9).

D. discoideum slug phototaxis operates via a lens effect. This means that parallel light which strikes the curved surface of the slug is focused, as a result of the increased index of refraction, onto the distal side of the slug. The slug then crawls away from the area of highest light intensity, i.e., toward the light source. Francis first showed this by illuminating, from above, only half of a slug's apex (6). The slug turned away from the illuminated side. If the slugs are placed in a medium with an index of refraction greater than that of the slug, the medium will cause the light to be dispersed as it strikes the slug. When Bonner and Whitfield did this, the slugs turned away from the light source (10). This type of result is typical of photoresponses which operate via a lens effect (11).

Several possible transduction mechanisms for slug phototaxis have been proposed. Bonner et al. suggested that slug phototaxis is just a special case of slug thermotaxis (5). This has been shown to be incorrect and the experiments which demonstrate this are discussed in the introduction to Chapter 5. Francis proposed several other possible transduction pathways: 1. that slug phototaxis is wholly an amoebal response, 2. amobae on the slug's distal side move more quickly than those on the proximal side

because of the greater light intensity or 3. light increases the resistance of the slime and the slug turns to the area where movement is least resisted (6). Poff and Loomis have reported a light-stimulated increase in migration rate and have proposed that the second transduction mechanism suggested by Francis is correct (12). However, with a slightly different technique Smith et al. were not able to observe the light stimulation (13). At this point, this discrepancy has not been explained. Fisher et al. have postulated that light generates a gradient of a slug repellent across the slug and the slugs actually respond to this gradient (see slug chemotaxis) (14). So far this hypothesis has not been tested adequately and the transduction pathway which operates in slug phototaxis is still unknown.

Recently Fisher and Williams have reported that phototactic mutants of D. discoideum exhibit bidirectional phototaxis (15). These mutants do not crawl directly toward the light, but at some discrete angle with respect to the incident light. Factors which tend to weaken the slug's phototactic response, increase this angle. These results, and some of my own, are discussed in Chapter 5 and Appendix B.

Slug Thermotaxis

In 1940 Raper reported that if slugs are exposed to a temperature gradient, they will crawl toward the warmer temperature (2). This directed migration on a temperature gradient is called thermotaxis. In 1950, Bonner et al.

studied the effect of stimulus strength on the slug thermotactic response (5). They reported that on gradients as weak as 0.028 °C/cm, the slugs will still tend to migrate toward the warmer side and that the thermotactic response reaches its maximum on gradients of around 0.06 °C/cm with about 90% of the slugs migrating toward the warmer temperature. Increasing the stimulus strength above this level did not alter the level of the response. Poff and Skokut confirmed these results and because of the extreme sensitivity of the response concluded that there was a specific receptor involved in slug thermotaxis, i.e., a general effect of temperature on metabolism would not allow a slug to respond to such weak temperature gradients (16).

Poff and Skokut measured the dependence of thermotaxis on the gradient midpoint temperature (16). Amoebae were grown and allowed to develop into slugs at 23.5°C and then placed on temperature gradient; if the gradient midpoint temperature was in the range of 22°C to 28°C a thermotactic response was evident. If the amoebae were grown at 20°C, instead of 23.5°C, the temperature range of thermotaxis was shifted to lower temperatures. Based on this adaptation and on the extreme sensitivity of the response, Poff and Skokut proposed that membrane lipids are involved in thermotransduction.

In 1980, Whitaker and Poff reported that if the gradient midpoint temperature was more than 1 to 3 degrees below growth and development temperature, the slugs would

exhibit negative thermotaxis, i.e., directed migration toward the cooler side of the gradient (17). The temperature, at which the transition from negative to positive thermotaxis occurred, was also dependent on the strength of the temperature gradient (17,18). These observations led Whitaker and Poff to propose that there are three sensors involved in D. discoideum slug thermotaxis, one sensor to mediate positive thermotaxis, the second to mediate negative thermotaxis and the third to control adaptation (17,18).

Whitaker and Poff examined the kinetics of the adaptation which occurs during development (18). They found the highest rate of thermal adaptation in hours 9 to 13 in a 15 hour development period. If a shift in temperature was made before the final 8 hours of development, the response was the same as if the amoebae were allowed to develop at new temperature for the total development period. During this critical time for adaptation, the amoebae are forming tight aggregates (unpublished observation) and many metabolic changes are taking place (19,20).

Whitaker also examined the effect of temperature on lipid content of amoebae (21). He found that after vegetative growth at 18°C instead of 23.5°C, the fatty acids from the amoebae grown at 18°C had an increased level of unsaturation with a decrease in the number of cyclopropane fatty acids. After these amoebae were allowed to develop into slugs, at their respective growth temperatures, the

differences in the fatty acid composition remained. Upon comparison with their 23.5°C counterparts, the amoebae which grew and developed at 18°C showed a decrease in the percentage of cyclopropane-containing fatty acids, a slight decrease in the amount of monounsaturated fatty acids and an increase in the percentage of diunsaturated fatty acids. Based on these studies, Whitaker and Poff proposed that the ambient temperature modulates the activity of a membrane-bound desaturase and therefore influences membrane fluidity (17). They suggested that this altered membrane fluidity has a different effect on the temperature-dependence of positive and negative thermotaxis and thereby shifts the transition temperature.

There has been a proposal which attempts to explain how a temperature difference across a slug results in directed migration. Loomis proposed that the warmer side of the slug experiences a lower relative humidity and therefore the slime sheath on that side provides more resistance to movement (4). Retarded movement on this side of the slug would cause the slug to turn toward the warmer side of the gradient. Subsequent measurements showed that the migration speed of a slug was temperature independent in the 22°C to 28°C temperature range (16). However, the most convincing argument against Loomis's proposal is its inability to explain negative thermotaxis.

In summary, slugs of D. discoideum are capable of positive and negative thermotaxis. The growth/development

temperature determines which response is expressed. The sensitivity of slugs to very weak gradients suggests a specific thermal sensor and three sensors have been invoked in an attempt to explain all the observed phenomena. One sensor is thought to control positive thermotaxis, another negative thermotaxis and a third to regulate adaptation.

Slug Chemotaxis

Fisher et al. have reported that slugs secrete a low molecular weight compound which they have called slug turning factor (STF) (14). As its name suggests, this compound acts as a repellent and the slugs respond to a STF gradient by turning away from the direction of higher concentration. Because light stimulates STF production and high concentrations of STF will interfer with slug phototaxis and thermotaxis, Fisher et al. have proposed that this molecule may be involved in these transduction pathways. However, using the methods of Fisher et al., I have been unable to reproduce their results on another strain of D. discoideum, strain NC-4, (unpublished) which is capable of thermotaxis and phototaxis (6,17).

Amoebal Phototaxis

Häder and Poff have recently demonstrated that amoebae of D. discoideum, strain AX-2, will either accumulate in or avoid a light trap depending on the intensity of the light and the duration of the irradiation (22,23). At light intensities of 3 to 10 W/m^2, amoebae will always accumulate in the light trap (22). At 20 to 100 W/m^2 the

amoebae will begin to accumulate in the trap, but after 30 to 90 minutes of irradiation the amoebae will disperse (22,23). With light intensities of 100 to 1000 W/m^2, the amoebae always move away from the light trap (23). The action spectra for the accumulation and dispersal are similar (22,23) which suggests that both responses utilize the same photoreceptor pigment(s). Both responses have an action maximum around 405 nm and secondary maxima at 580 and 640 nm. There is also a broad band of activity which encompasses the green and long-wavelength blue (22,23). The low temperature absorption spectrum of the amoebae has bands at these maxima (23), and these bands, in part, are thought to be the result of protoporphyrin absorption (J.C. Lagarius, personal communication). Even though amoebal photoaccumulation and photodispersal probably utilize the same receptor, Häder and Poff have been able to separate these two responses with ionophores and a protonophore (24). These results suggest differences in the transduction pathways.

By examining individual amoebae, it was determined that accumulation in the light trap was the result of positive phototaxis toward the light scattered by the amoebae already in the trap (22). Dispersal was the result of a negative phototctic response to the scattered light (23). Because the amoebae did not respond to infrared light, Häder and Poff concluded that these responses were truly

light-stimulated and not the result of a light-generated temperature gradient (22,23).

Hong et al. were able to obtain a more direct measurement of positive and negative amoebal phototaxis (25), and the resultant action spectrum resembled those determined with the light trap. These action spectra suggest that slug phototaxis can not be totally explained on the basis of amoebal phototaxis because all these action spectra which characterize the amoebal photoresponses do not resemble the action spectrum for slug phototaxis (6).

Amoebal Thermotaxis

Amoebal thermotaxis has recently been described (C. Hong, personal communication). This response is weaker than that of the slug and appears to change as development proceeds. Upon starvation, the amoebae are only capable of positive thermotaxis and, as they develop, they gain the capacity for negative thermotaxis. The resulting temperature-dependence of thermotaxis, the response of thermotactic mutants, and the adaptation to a changed ambient temperature are so similar in the slug and the amoebae that is has been suggested that slug thermotaxis is a composite of the amoebal response.

Amoebal Chemotaxis

In D. discoideum, the best characterized sensory response is amoebal chemotaxis and this is because the chemotactic response to cAMP plays a major role in development. However, the amoebae do respond

chemotactically to other compounds, and one of these is folic acid (26). Folic acid is secreted by the bacterial food source, and vegetative amoebae, not aggregating amoebae, respond to it by crawling toward areas where it is present in a higher concentration (26,27). For these reasons it has been proposed that this positive chemotactic response is a means of food detection (26,27).

As in the case of slugs, amoebae produce a small molecular weight compound which repels other amoebae (28,29,30). The repellant seems to be a small molecular weight metabolite produced by vegetative amoebae, but preaggregative as well as vegetative cells respond to it (29,30). It has been proposed that this negative chemotactic behavior aids in amoebal dispersal (28,30).

After interphase, amoebae are able to respond to cAMP by positive chemotaxis and by producing and releasing their own cAMP. By alternating periods of receptiveness with refractive periods, the amoebae are able to aggregate and form large mounds. For reviews see references (4,31).

There has been progress in elucidating the cAMP transduction pathway. Work with inhibitors and mutants have implicated cGMP as the mediator of the cAMP response. For a review see reference (32). The addition of cAMP causes an immediate, but transient, 10 to 20-fold increase in cGMP. This increase appears to enhance the activity of a phospholipid methyltransferase and thereby causes an increase in the amount of phosphatidylcholine in the amoebal

membranes (33). Alemany et al. have proposed that the altered membrane composition is reflected in the activity of a membrane-bound enzyme which transports Ca^{2+} thus increasing the local intracellular Ca^{2+} concentration (33). The increase in Ca^{2+}, with the aid of calmodulin, is thought to inhibit myosin heavy chain kinase. The reduced phosphorylation of myosin would increase the activity of an actin-activated ATPase and stimulate the self-assembly of myosin into filaments. For a review see reference (34). This whole series of events would lead to pseudopod formation, which is the first visible step in an amoebal chemotactic response (35).

The addition of cAMP also results in the methylation of a 120,000 molecular weight protein (36), the acidification of the medium (37,38), and an increase in the number of smooth vesicles thought to be involved in exocytosis (39). Gerish has proposed that these vesicles contain the cAMP to be secreted (39) and the pH change is thought to be associated with the adaptation to cAMP which causes the refraction period (34). Whether the proposed transduction mechanism is correct and what role these other events play remains to be determined.

CHAPTER 1

General Materials and Methods

Growth and Development of Dictyostelium discoideum.

Dictyostelium discoideum amoebae strains NC-4, HL50, and mutants derived from HL50 were grown on solid medium in association with Klebsiella aerogenes as described by Sussman (40). Amoebae of the axenic strain, AX-2, were grown in liquid culture on a rotary shaker in a previously described medium (22). All amoebae were grown in darkness at 23.5 $\pm$ 0.3°C unless otherwise stated.

The strains of D. discoideum grown in association with bacteria were harvested by washing the agar with potassium phosphate buffer (15 mM, pH 6.1). The amoebae were washed three to four times with buffer in order to remove the remaining bacteria. Amoebae of strain AX-2 were harvested during exponential growth by centrifugation and washed twice with phosphate buffer to remove the nutrient medium. Centrifugations of one to two minutes at 500 x g were always used. The amoebae were washed once with double distilled water and suspended in the distilled water so that the final volume was 3 x 10^8 amoebae per ml. A 10 µl droplet of this suspension was placed in the center of a Petri dish containing non-nutrient water agar (2%, w/v). Slugs were

allowed to form in darkness at 23.5 ± 0.3°C unless otherwise stated. The Petri dishes were placed on a thermal gradient for thermotaxis experiments or exposed to unilateral light for a phototaxis experiment.

Thermal Gradients and Phototaxis Apparatus

The thermal gradients used in the following experiments were similar to those described by Poff and Skokut (16). They consisted of an aluminum slab, approximately 9 cm wide, which had two conduits drilled it in such that the conduits ran along the length of the slab. The conduits were connected to circulating water baths (Model 2095, Forma Scientific, Marietta, OH; RTE-8, Neslab, Portsmouth, NH; Lauda NDB8/17, Brinkmann Instruments, Inc., Westbury, NY). By varying the temperature of the water baths, a thermal gradient could be generated across the slab. The midpoint temperature and the steepness of the thermal gradient could also be adjusted. The aluminum slabs were set in insulated coolers such that, except for the connecting water lines, they were surrounded by styrofoam. Approximately ten minutes after the coolers were closed, the temperature gradient stabilized. The coolers and attached water baths were placed in a dark, temperature-controlled room. If the water baths were set at the same temperature, the D. discoideum slugs on the aluminum slab migrated in a random manner.

The gradient temperatures were measured by determining the potential which resulted from the placement of a

copper-constantan junction at one point on the gradient with its reference copper-constantan junction at another. The potential could be converted into the temperature difference between the two ends of the constantan wire (Figure 1). The error in the measurement was less than 0.2°C or 0.02°C/cm on the gradients used.

Since much of the energy, in the form of heat, was being expended just to maintain the aluminum slab at the appropriate temperature, a gradient was made out of a hollowed slab. This gradient consisted of the two conduits connected by a sheet of aluminum. The Petri dishes sat on this sheet. The temperature across the gradients was measured as a function of the temperature difference between the water baths (Figure 2). The temperature of the water baths was measured with a mercury-in-glass thermometer accurate to less than 0.1°C. Because the temperature difference across the non-hollowed slab appeared to begin to saturate when a relatively small temperature gradient was applied across it, this gradient and others like it were used only when the temperature difference between the water baths was 2°C or less. When measurements were taken at various points across a temperature gradient, the gradients appeared linear.

For the phototaxis experiments, the light source was a Leitz Prado slide projector equipped with a Baird-Atomic interference filter (428 or 430 nm with a half-band pass of 10 nm). The intensity of the light was controlled by

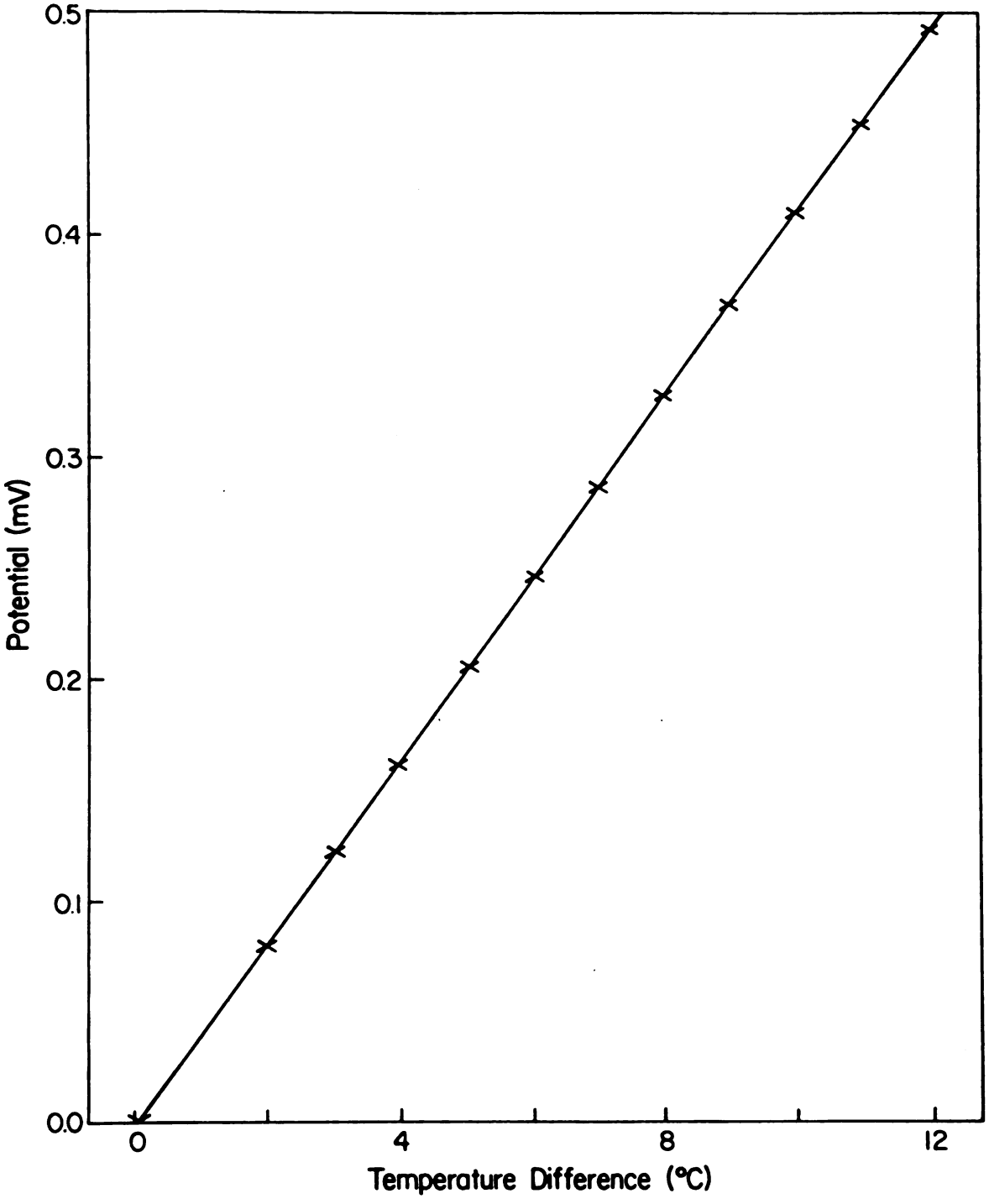

Figure 1. Calibration curve which was used to convert the measured potential into the temperature difference.

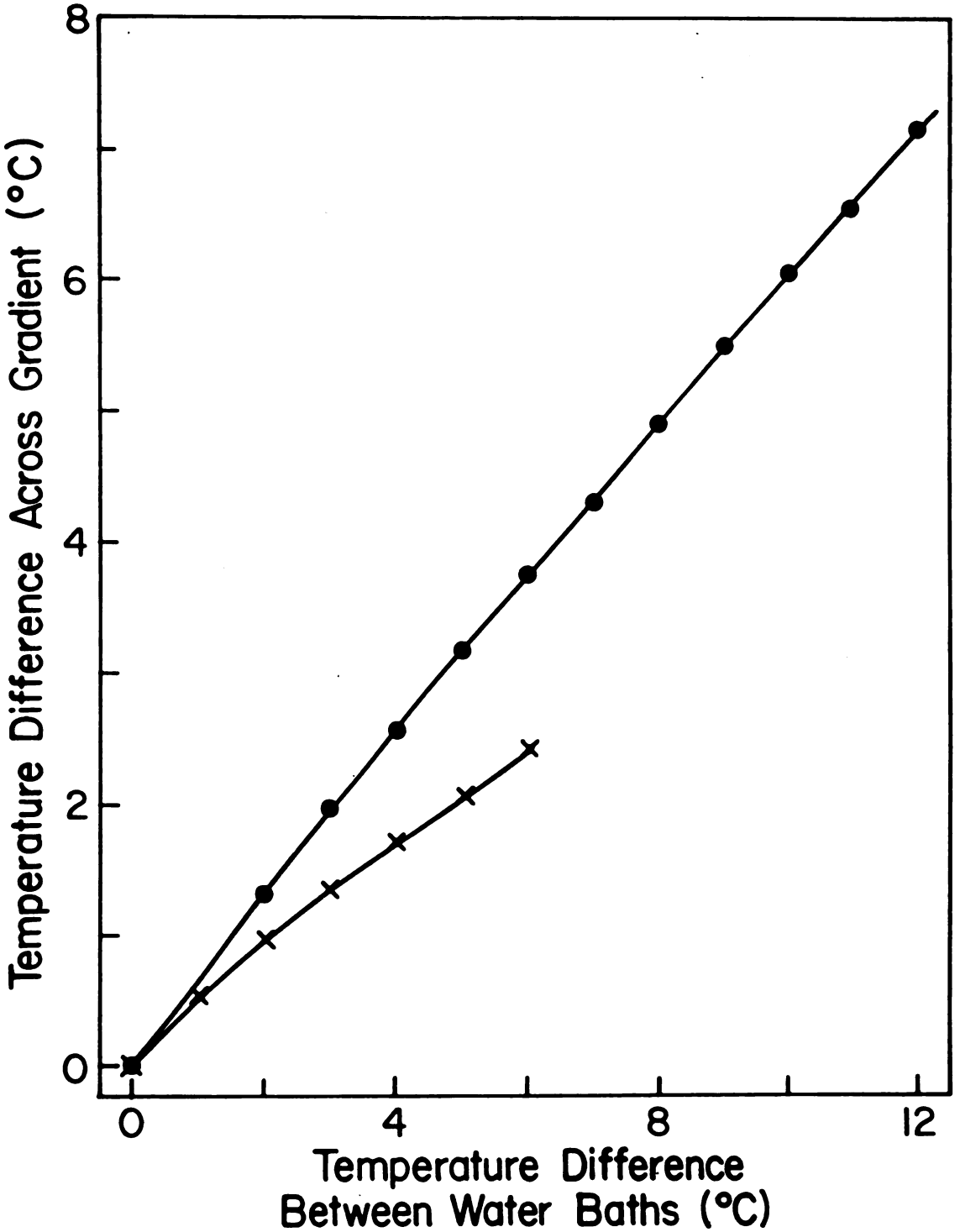

Figure 2. Temperature difference across a 9 cm gradient as a function of the temperature difference between the water baths. ●—● , hollowed slab; X—X, non-hollowed slab.

stacking neutral density filters (Röhm and Haas, gray 838, plexiglass, 3.0 nm thick). The light intensity was measured with an International Light Spectroradiometer (Newburyport, Mass). The phototaxis experiments were conducted in a dark, temperature-controlled room at 23.5 $\pm$ 0.3°C unless otherwise stated.

Directness of Migration

Several methods have been employed to quantify the response of a population of slugs to an environmental stimulus. Bonner et al. used the percentage of slugs on the warmer side of the gradient to represent the degree of response (5). These authors, in assaying for phototaxis, measured the angle of orientation with respect to the light source for each slug. Poff and Skokut used 360° minus the angle which encompassed all the slugs and their slime trails as a measure of slug thermotaxis (16).

Recently two other methods of quantitation have appeared (14,17). These two calculations are mathematically rigorous and are interrelated. The first, and most basic, is based on an empirical circular distribution, i.e., each slug may be represented by a point on the circumference of a circle. Assuming a unit circle, the angle (δ) and the length (r) of the mean vector is calculated in the following manner:

$$x = \frac{1}{n} \sum_{i=1}^{n} \cos a_i$$

$$y = \frac{1}{n} \sum_{i=1}^{n} \sin a_i$$

$$r = \sqrt{x^2 + y^2}$$

where a_i is the angle which represents the position of the slug. δ can be calculated from x,y, and r by:

$$\cos \delta = \frac{x}{r}$$

$$\sin \delta = \frac{y}{r} .$$

The statistic, r, is used to quantify directness of migration by the slug population. Because the calculation is based on the unit circle, r varies from zero to one with zero denoting a random population and one representing a 'perfectly directed' population (41).

The other calculation which is currently used to describe the migration of a population of slugs is based on a circular normal distribution (41). This distribution assumes the following density function

$$f(a) = \frac{1}{2\pi I_o(K)} e^{K \cos (a-\theta)}$$

where θ is the angle of maximum density, K is a parameter related to the concentration of slugs along the mean direction of migration, and I_o (K) is a hyperbolic Bessel function. The parameter, K, is used to quantify slug response and is related to r by the following

$$r = \frac{I_1 (K)}{I_o (K)}$$

where $$I_1(K) = \frac{d}{dK} I_o (K).$$

Practically, r is calculated from the data and then the conversion from r to K is achieved via simplified equations which are only valid for certain ranges of r or the conversion is achieved by consulting a table (41,42). The values of K vary from zero for a random population to infinity for a 'perfectly directed' population.

Neither r nor K is an ideal measure of the directness of migration of a population of slugs. The statistic r is sensitive to relatively weak responses, but saturates at around 0.95 when the responses become rather directed (Figure 3). The actual point of saturation is dependent on the distribution of the slugs. The parameter K is based on a model probability distribution and it has never been shown that this distribution correctly describes the migration of a population of D. discoideum slugs. This parameter is also insensitive to less directed populations and in more directed populations varies dramatically with only slight increases in directness (Figure 3).

In this dissertation, the statistic r will be used. Because there is no simple relationship between r and K and because K is determined entirely from r and therefore contains no additional information, K will be avoided.

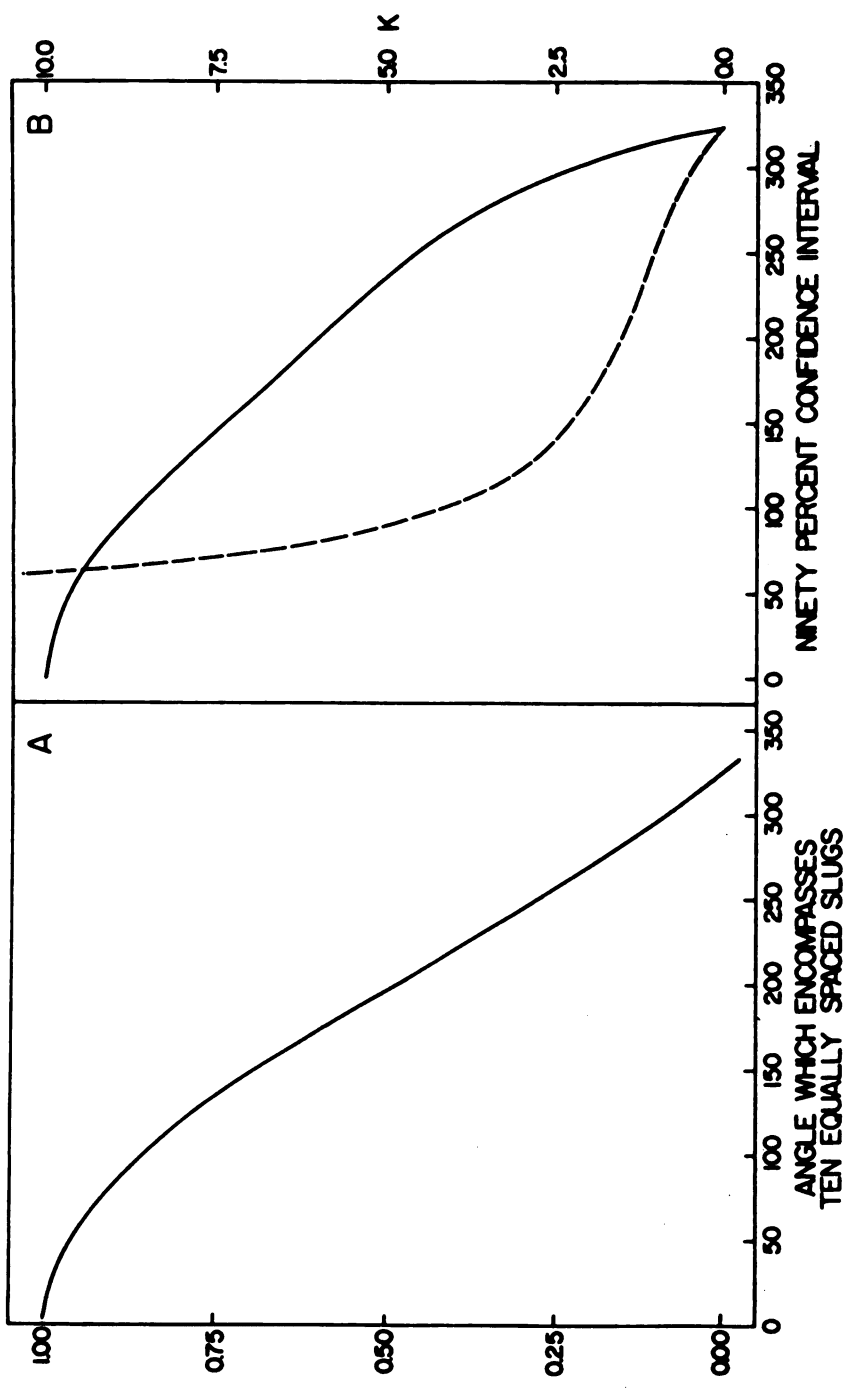

Figure 3. The statistics, r —— and K - - -, plotted as a function of slug distribution. In A ten slugs were equally spaced and in B a circular normal distribution was assumed.

General Observations

There are several factors which have been found to influence the apparent directness of D. discoideum slug responses. The first is a result of the directional statistic just described. Because only the final positions of the slugs are used to determine directness, the time at which the experiment is ended can be critical. This is especially important if the slugs change direction of preferred migration during the course of an experiment, which frequently happens around the transition temperatures in thermotaxis experiments. In an attempt to circumvent this problem, the slugs were allowed to crawl at least 3 cm before any experiment was ended. This is why the length of the experiments varied from 24 to 48 hours. Also, if a slug stopped crawling and formed a fruiting body before it had crawled the 3 cm, it was not included in the analysis.

The position of the D. discoideum slugs when placed on the thermal gradient or in the phototaxis apparatus can also influence the resultant r value (Figure 4). The results shown indicate that the Petri plates can be placed on the thermal gradient at any point past the tight aggregate stage, but that they must be placed on the gradient before the slugs begin to crawl away from the innoculum spot. Once the slugs have begun to migrate, they show a reduced tendency to turn (unpublished observation); therefore their response to an environmental stimulus is weakened. The strength of the thermotactic or phototactic response will

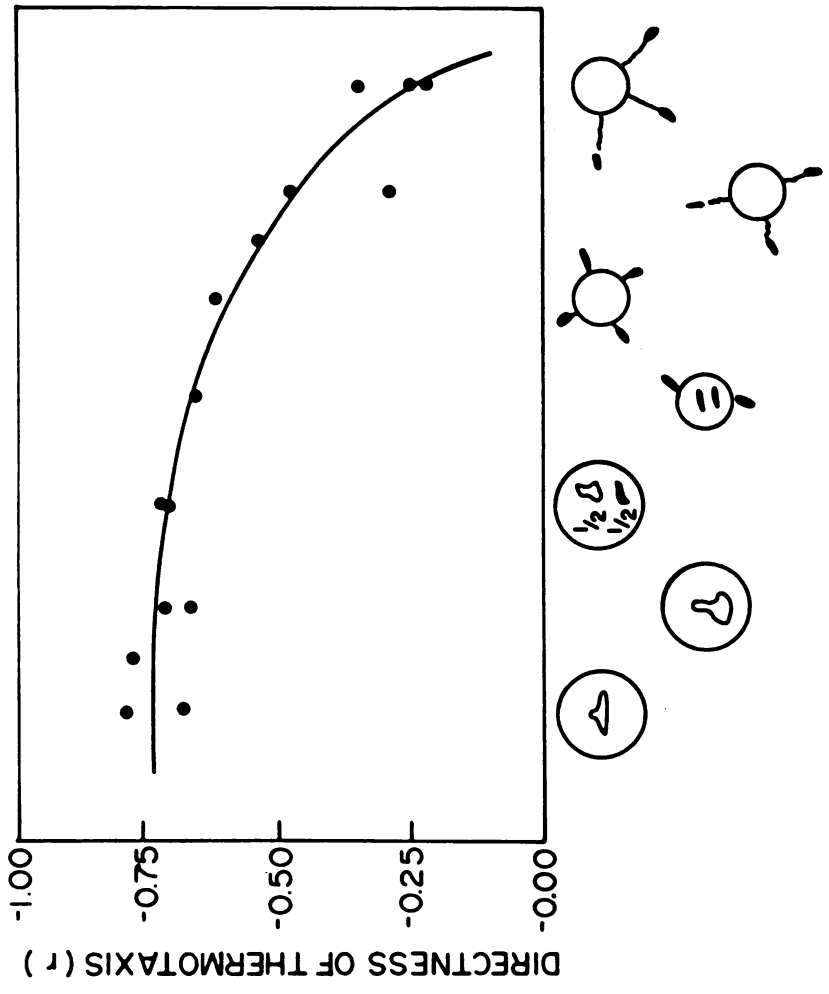

Figure 4. Thermotactic response of D. discoideum strain NC-4 as a function of developmental stage. The gradient was a 0.11 °C/cm gradient with a midpoint temperature of 17°C.

dictate how far, if any, the slugs can crawl before placement on the thermal gradient or in the phototaxis apparatus before the value of r is affected.

Another factor which is important in the thermotaxis experiments is the fine layer of water which is used to improve the contact between the aluminum slab and the Petri plates. In the absence of this water film, the resultant r value can drop about 10%.

CHAPTER 2

Role of Bulk Lipid Fluidity in the Thermal Adaptation of *Dictyostelium discoideum* Thermotaxis

Introduction

The amoebae of *Dictyostelium discoideum*, a cellular slime mold, feed on bacteria associated with decaying matter. Upon depletion of their food source, the amoebae aggregate and form a pseudoplasmodium or slug. The slug is capable of negative or positive thermotaxis, i.e. directed migration toward the cooler (negative) or warmer (positive) side of a temperature gradient (17). Whether negative or positive thermotaxis is expressed depends on the temperature of amoebal growth and development into slugs (17,18). This dependence of the response on the temperature history of the amoebae/slug is called adaptation.

It was postulated by Poff and Skokut that a lipid or lipids in the membrane matrix might be acting as the temperature sensor(s) (16). This hypothesis was based on the high sensitivity of the response (5,16) and the existence of thermal adaptation (16). Whitaker and Poff proposed that thermal adaptation might involve the "restructuring of membrane lipids" and that the temperature sensor that controls adaptation might be an enzyme involved

in lipid metabolism (17). A temperature-controlled desaturase, whose activity would alter membrane fuidity, was suggested.

All the preceding results were obtained with the wild type strain of Dictyostelium discoideum, NC-4. Recently Mohan Das et al. reported that amoebae of D. discoideum, strain AX-2, when grown at different temperatures, exhibit no systematic change in fatty acid composition or sterol:phospholipid ratio (43). They also found no effect of growth temperature on the fluidity of the membranes of these amoebae, measured using electron spin resonance (ESR) and the probe 5-doxyl stearate (5-DS).

The report of Mohan Das et al. raises several questions with regard to D. discoideum thermotaxis and the proposed mechanism for thermal adaptation. 1. Does strain AX-2 show thermotactic responses similar to those exhibited by strain NC-4? 2. Are these responses dependent upon the amoebal growth temperature? 3. Are the responses of strain AX-2 altered by changing the temperature at which development occurs? 4. Does the lipid fluidity change if the developmental temperature is changed? In this chapter, I address these questions.

Materials and Methods

An axenic strain of Dictyostelium discoideum, AX-2, was grown and harvested as described in Chapter 1. The growth and development temperatures were either 18°C or 23.5°C. Thermotaxis experiments were conducted and the directness of

the response was calculated as described. The gradient strength was 0.11°C/cm.

For ESR experiments, 10 µl droplets of the amoebal suspension were placed on non-nutrient water agar (2%, w/v). The amoebae were incubated, in darkness, at 18°C or 23.5°C until 90% of the aggregates had formed slugs. The aggregates were washed off the agar with phosphate buffer, disrupted by vigorous pipetting, and the suspension washed with buffer. The lipids were extracted with a modified Bligh and Dyer procedure (44) and possible remaining proteins removed as described by Wuthier (45). The lipids were spin-labeled with 5-doxyl stearate and the ESR experiments performed and analyzed as described by Fontana and Haug (46). Briefly, the spin label, S-DS (Synvar Crop., Palo Alto, CA), was added to an aqueous suspension of lipids such that the final concentration of spin label to lipid was less than 0.2% (w/w). The lipid suspension was then sonicated for 10 minutes before being placed in the ESR cuvette. The spectrometer used in these experiments was a Varian X-band, model E-112, with an attached temperature controller. The ESR spectra were recorded after allowing a five minute equilibration at each temperature. The analysis involved measuring the hyperfine splitting parameter ($2T_{||}$) and calculating the order parameter. The hyperfine splitting parameter is inversely related to lipid fluidity and the order parameter is a relative measure of the

deviation of a fatty acyl chain from a position normal to the plane of the membrane.

Results

Slugs of D. discoideum, strain AX-2, are capable of both positive and negative thermotaxis (Figure 5). When the amoebae are grown and develop into slugs at 23.5°C, the transition from negative to positive thermotaxis occurs around 20.5°C. This transition temperature is the same as that observed with D. discoideum strain NC-4 (17).

In strain AX-2, as in strain NC-4, amoebal growth temperature influences the thermotactic response of the slug (Table 1). The growth temperature affects not only the strength of the thermotactic response, but can also affect the direction of movement (positive or negative thermotaxis).

The temperature at which the slug formation occurs is also critical in determining response (Figure 5). With an amoebal growth temperature of 23.5°C, the transition temperature from negative to positive thermotaxis is shifted from 20.5°C for slugs developed at 23.5°C to 17.5°C for slugs developed at 18°C.

These results show that strain AX-2 is capable of thermotactic responses similar to those seen with strain NC-4, and that these responses are dependent on the temperature at which amoebal growth and development proceeds. Mohan Das et al. have already shown that altering amoebal growth temperature does not alter membrane fluidity

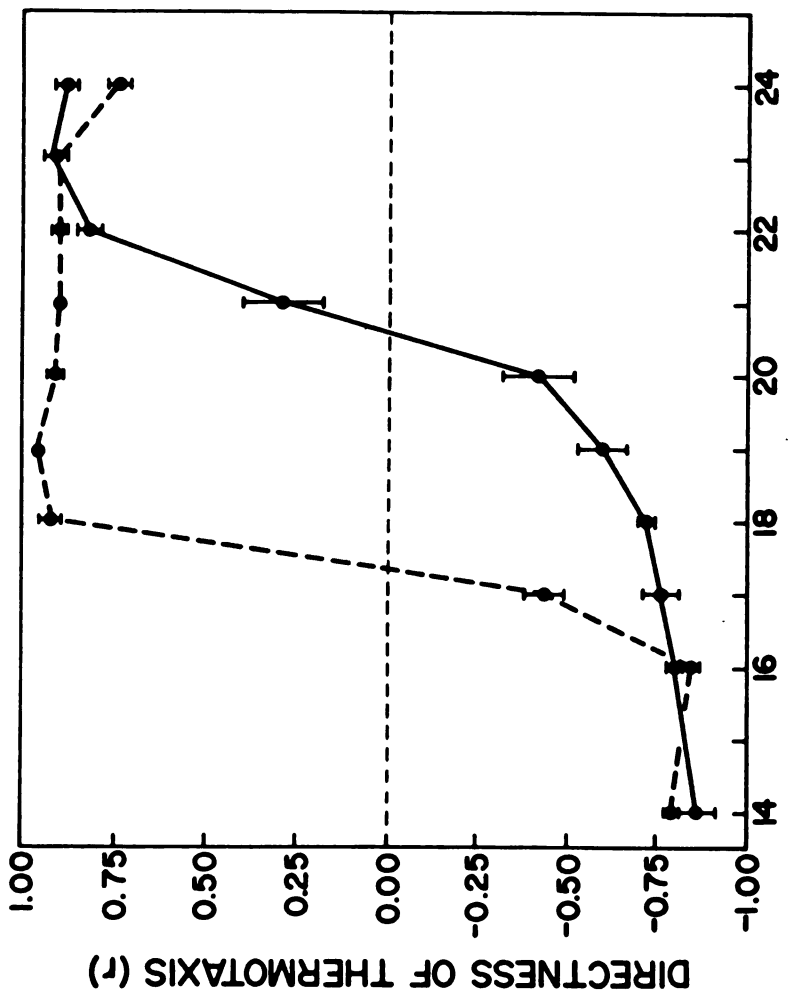

Figure 5. Temperature response curves of strain AX-2 grown at 23.5°C and developed into slugs at 18°C (- -) and 23.5°C (—). Each point is the average of at least five separate experiments. The vertical bars represent ± one standard error of the mean.

Table 1. Dependence of thermotactic response on amoebal growth temperature.

AMOEBAL GROWTH TEMPERATURE	DEVELOPMENT 18°C GRADIENT MIDPOINT 17°C	DEVELOPMENT 23.5°C GRADIENT MIDPOINT 19°C
18°	0.58 ± 0.07[1]	0.20 ± 0.12
23.5°C	-0.44 ± 0.05	-0.60 ± 0.07

1. The values given are directness of response ± 1 SEM. Each value is the average of six determinations.

as determined with ESR and 5-DS (43). However, the Whitaker and Poff hypothesis (17) suggests that changing growth or development temperature should alter lipid fluidity. Therefore, the effect of development temperature on lipid fluidity was tested.

The hyperfine splitting ($2T_{||}$), was linearly related to temperature in the range of 0°C to 28°C ($r = 0.98$, Figure 6). The order parameter also varied linearly with temperature ($r = 0.96$, data not shown). Thus, it appears that there are no major bulk lipid phase changes in the physiologically relevant temperature region.

The values obtained for the hyperfine splitting (Figure 6) and the order parameter were independent of development temperature. At a gradient midpoint temperature of 18°C, a slug which was formed at 18°C would have a directness of thermotaxis of 0.92 while a slug formed at 23.5°C would have a directness of -0.72. This very large difference in response occurs with no observable difference in lipid fluidity. These results suggest that bulk lipid fluidity does not determine the thermotactic response in D. discoideum and is not involved in thermal adaptation.

Discussion

The search for a chemoreceptor or photoreceptor is simplified by the fact that only a few biological molecules are capable of interacting with the stimulus. In contrast, every biological molecule responds to a temperature change. Therefore, when searching for the thermal sensor or

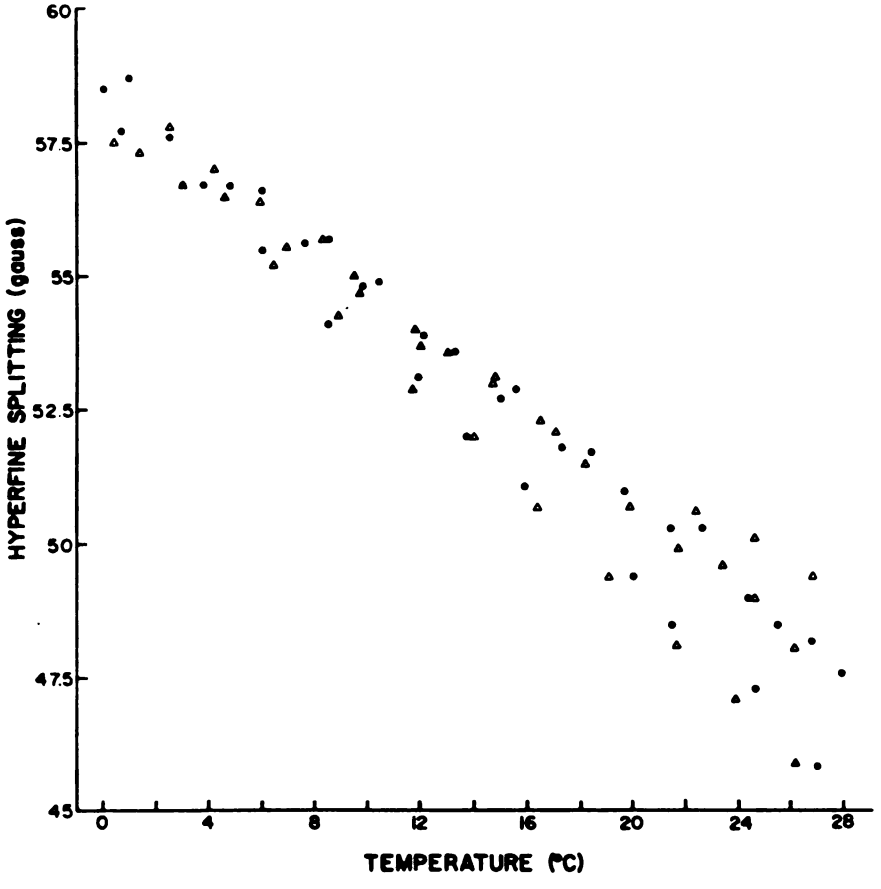

Figure 6. Hyperfine splitting ($2T_{||}$) of lipids extracted from slugs developed at 18°C (●) and 23.5°C (Δ). For each development temperature, data from three different extractions are given.

receptor, other characteristics of the thermal response must be examined. The sensitivity and adaptability of the thermotactic response in D. discoideum suggest membranes or components of them as candidates for the sensor.

If one assumes a membrane is involved, it can not be assumed a priori which membrane is involved. The effect of growth temperature on fatty acid composition and membrane fluidity was studied with a crude membrane fraction (43). In order to eliminate possible protein effects, this study dealt with extracted lipids. Both sample types resulted in the same conclusion, i.e., changing the temperature history of D. discoideum does not alter the bulk fluidity of its lipids. These results are of interest because in plants, where changing the ambient temperature alters a physiological response, methods such as those described here have been used to correlate lipid fluidity and the response (47).

Herring et al. examined the plasma membrane of D. discoideum, strain AX-2, and found that substituting up to 53% of the fatty acids with polyunsaturated fatty acids did not alter the fluidity of this membrane as determined by electron spin resonance and fluorescence depolarization (48,49). They also used ESR to examined the phosphatidylcholine molecules and found no effect of fatty acid substitution on their fluidity. Based on these results they suggested that a membrane, such as the plasma membrane of D. discoideum, which contains a large number of unsaturated fatty acids does not become significantly more

fluid when this number is increased (49). This lack of sensitivity to altered composition again implies that bulk lipid fluidity is not involved in the thermal adaptation of the D. discoideum thermotactic responses.

The lack of adaptation in bulk lipid fluidity does not eliminate membrane components as the thermal sensor or a change in membrane composition being involved in thermal adaptation. It is possible that changing the temperature during growth or development alters a specific membrane component and this component in turn alters the thermotactic response. The measurements reported here and those of Mohan Das et al. (43) would not have detected such a subtle localized change in a particular lipid. It is equally possible that bulk lipid fluidity is the sensor for a temperature difference across a slug and that thermal adaptation involves another step in the sensory transduction pathway.

Summary

Thermotactic responses of Dictyostelium discoideum, strain AX-2, were examined and found to be similar to those of strain NC-4. These responses, like those of strain NC-4, were dependent on the temperature at which the amoebae grew and developed into slugs. The lipid fluidity of strain AX-2 was found to be independent of development temperature. Consequently, the hypothesis that a change in bulk lipid fluidity is the mechanism for the adaptation of D. discoideum thermotaxis appears to be untenable.

CHAPTER 3

Mutants of Thermotaxis in Dictyostelium discoideum

Introduction

Dictyostelium discoideum is a cellular slime mold which, as an amoeba, grows vegetatively utilizing bacteria as its food source. Upon starvation, the amoebae aggregate and form a multicellular mass called a pseudoplasmodium or slug. This mass is capable of directed migration in response to light (2,13), chemicals (14), and thermal gradients.

Thermotaxis was first observed by Raper (2). The extreme sensitivity of the response was reported by Bonner et al. (5). Poff and Skokut described the temperature range in which thermotaxis could occur and reported that the range was dependent on growth temperature (16). In extending these observations, Whitaker and Poff observed that D. discoideum is capable of directed migration toward the cooler side of a thermal gradient (negative thermotaxis) or to the warmer side (positive thermotaxis) (17). Growth and development temperature, gradient midpoint temperature and the strength of the gradient were factors in determining which response was expressed. Based on these results, Whitaker and Poff proposed that there are three

biothermometers in D. discoideum; one thermometer was thought to regulate the positive response, the other the negative response, and the third to regulate adaptation.

If this proposal of separate biothermometers is correct, then it should be possible to obtain mutants defective in one response and not the other. These mutants might also be useful in determining the molecular interactions involved in thermosensory transduction. For these reasons, a mutant selection was undertaken (50).

The mutants which are characterized in this chapter were generated by exposure of D. discoideum, strain HL50, to N-methyl-N'-nitro-nitrosoguanidine (50). A 99% kill rate was obtained. The thermotactic mutants were selected by visual inspection after migration for 24 hours on a 0.11 °C/cm thermal gradient with a midpoint temperature of 16°C or on a 0.06 °C/cm gradient with a midpoint of 23°C.

Materials and Methods

Dictyostelium discoideum strain HL50 and mutants derived from this strain were grown, harvested and allowed to develop as described (Chapter 1). The thermal gradients were set at 0.11 °C/cm with various midpoint temperatures. The slugs were allowed to migrate on these gradients for 24 to 48 hours. The Petri dishes were then removed from the thermal gradients and directness of thermotaxis was calculated (Chapter 1). The transition temperatures were determined by interpolation to the nearest 0.1°C from the temperature-response curves.

Results

Although most of the earlier work on D. discoideum thermotaxis was performed on strain NC-4, another strain, HL50, was considered as the parental strain for the mutant search. HL50 has the genetic markers Acra A on chromosome II and bsg B on chromosome VII. These markers permit selection of diploids and haploids in a parasexual genetic analysis. The temperature response curve for HL50 (Figure 7) has a similar shape to that reported by Whitaker and Poff for NC-4 (17). The major difference in the temperature response curves for HL50 and NC-4 is in their transition temperatures from negative to positive thermotaxis. For amoebae grown and allowed to aggregate at 23.5°C, the transition temperature is 18.7°C for HL50 and 20.5°C for NC-4. Because of the similar responses to temperature gradients, HL50 was deemed acceptable as the parental strain for this analysis.

In the first 1000 mutagenized clones which were screened, 8 were found with apparently altered thermotactic responses. Of these, several were unstable and others were eliminated because of an increased tendency to sporulate or because of very slow migration rates. Four clones in three classes appear to be stable and relatively unimpaired in other processes. One of the clones, HO428, did not exhibit negative thermotaxis (Figure 7A), but showed positive thermotaxis over its entire temperature range of migration. This mutant also has a more limited temperature range for

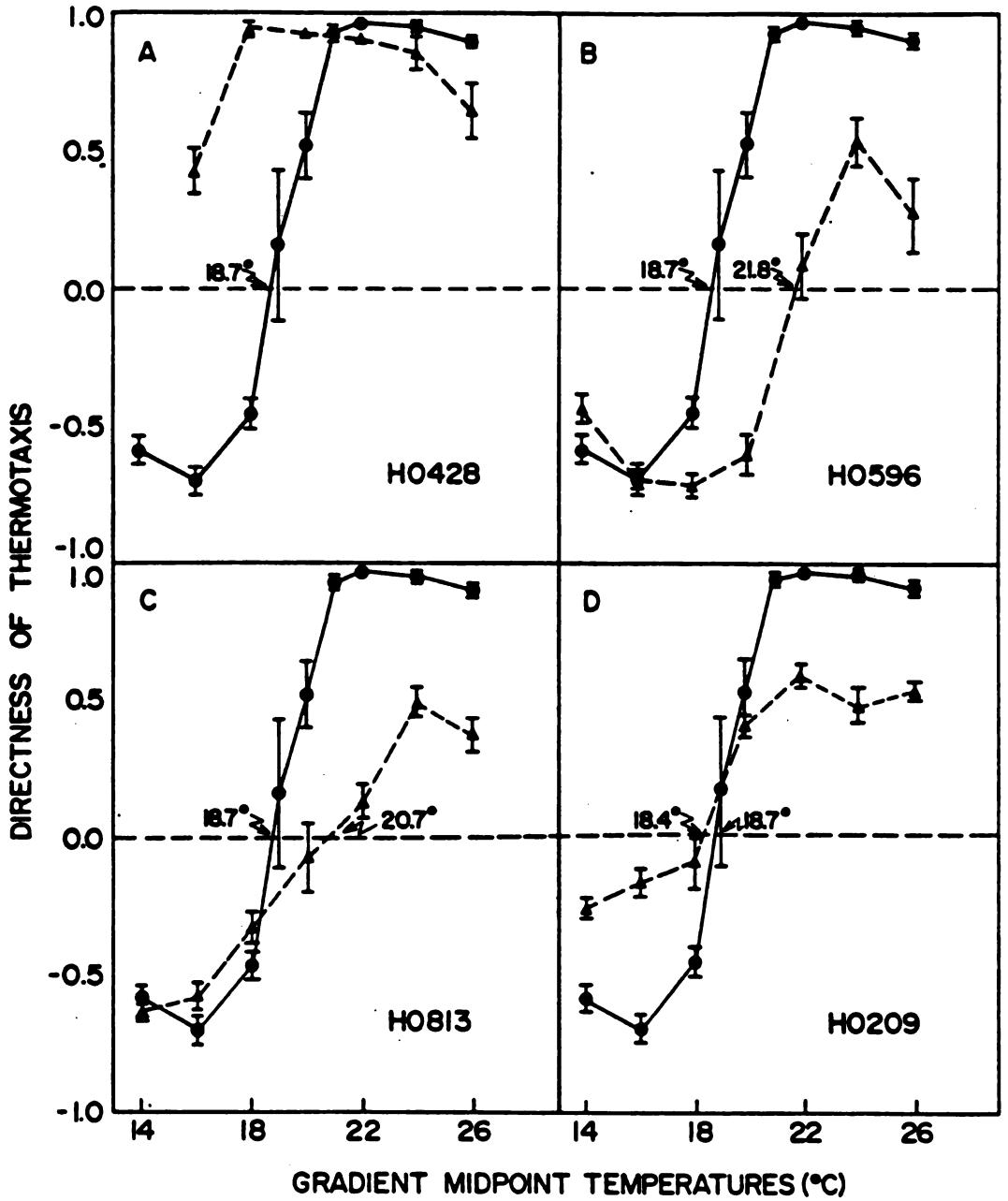

Figure 7. Temperature-response curves of HL50 and mutants of thermotaxis. The gradient strength was 0.11 °C/cm and the results of at least five separate experiments were used to determine each point. The vertical bars represent ± one standard error of the mean. ●—● , HL50; ▲--▲, mutants. (A) HL50 and H04281; (B) HL50 and H0596; (C) HL50 and H0813; (D) HL50 and H0209.

migration than HL50 or any of the other mutants as indicated by its inability to crawl at 14°C and its decreased migration at 26°C. The response of HO428 on a gradient which elicited a positive thermotactic response from HL50 suggests that the mutation has not blocked the sensory pathway which results in positive thermotaxis. No mutants have yet been found with a normal negative thermotactic response and no positive response.

Two other mutants, HO596 and HO813, demonstrated a reduced ability to respond on gradients where HL50 demonstrated positive thermotaxis (Figure 7B and 7C). The responses of HO596 and HO813 on gradients with midpoint temperatures of 22°C, 24°C, and 26°C were quantitatively equivalent. On gradients which usually produce a negative response with HL50, the response of HO813 appears to be the same as that of the parental strain. The weakened positive response in both mutants resulted in a shift toward a higher temperature in the transition between positive and negative thermotaxis. The transition of HL50 is at 18.7°C, while the transition of HO813 is at 20.7°C and that of HO596 is at 21.8°C. No mutant has been found with both a reduced negative thermotactic response and a normal positive response.

The last type of mutant found (HO209) showed a weakened positive and weakened negative thermotactic response (Figure 7D). The transition between positive and negative thermotaxis is approximately the same for this mutant as for

the parental strain. Shadowgraphs of these mutants on 0.11 °C/cm gradients, with gradient midpoint temperatures at 16°C and 22°C, clearly demonstrate the mutant phenotypes (Figure 8). In accordance with convention, mutants HO428, HO596, HO813, and HO209 have been designated thm 2000, thm 2001, thm 2002, and thm 2003 respectively.

Discussion

The mutants described in the preceding section support the proposal of Whitaker and Poff that positive and negative thermotaxis are separate responses. The mutants demonstrate the existence of genetic elements which, when altered, affect one thermotactic response and not the other. Mutant HO209 also suggests that positive and negative thermotaxis do have steps common to both pathways. However, in no case has it been demonstrated that the mutants described have just one mutation in the genes which control thermosensory transduction.

If positive and negative thermotaxis are the result of separable transduction pathways, as appears to be the case, then the transition from one response to the other is at the point where the two responses are approximately equal in strength. This point is called the transition point. At temperatures above the transition point the positive response dominates, and at temperature below the transition point the negative response dominates.

The shift in the transition points which is observed in the mutants, upon comparison with the parental strain, is

Figure 8. Redrawn shadowgraphs of Dictyostelium clones on 0.11 °C/cm temperature gradients with the upper side of the figure corresponding to the warmer side of the two gradients. Gradient midpoint temperatures were 16°C (A) and 22°C (B). The pseudoplasmodia can be seen at the ends of slime trails leading from the inoculum drop.

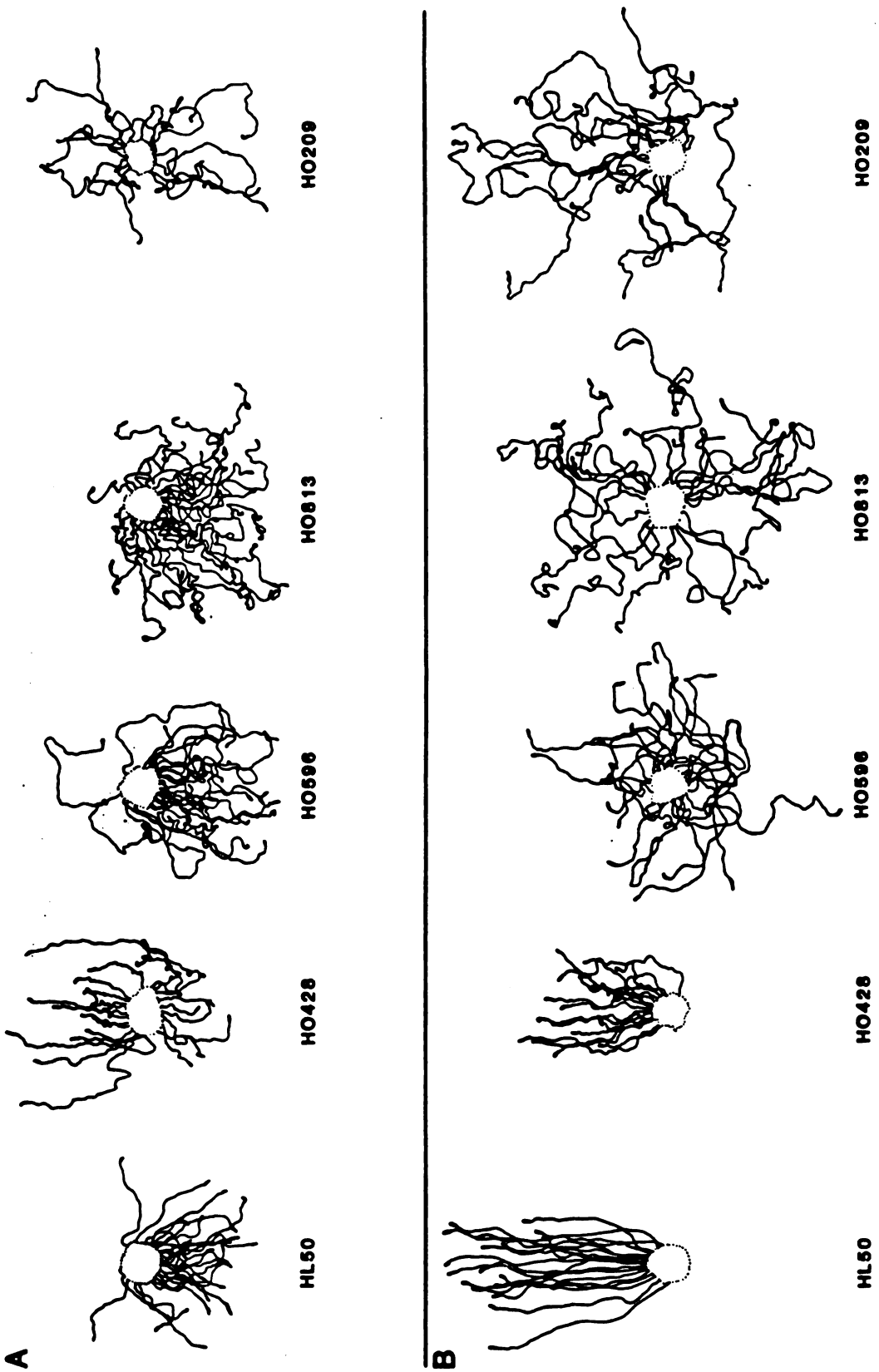
A
HL50
HO428
HO596
HO613
HO209
B
HL50
HO428
HO596
HO613
HO209

Figure 8

consistent with the separable responses interpretation. Mutant H0596 and H0813, which appear to have weakened positive responses and near normal negative response, have transition points which are shifted to higher midpoint temperatures. This suggests that the positive response, because it is weakened, must be at a temperature closer to its maximum before it can dominate. H0428, perhaps because of its inability to crawl at 14°C, does not appear to have a transition point. The temperature-response curves suggest that if H0428 does have a transition point, it is shifted to a temperature much lower than that of the parental strain. This is consistent with the normal positive thermotactic response of H0428 and the apparent absence of a negative response. Upon comparison of HL50 and H0209, the lack of an observable shift in the transition temperature is consistent with the approximately equal diminution of the positive and negative response. Thus, mutant H0209 appears to be defective in a downstream amplification step.

In the 1000 clones screened, a mutant totally lacking positive thermotaxis was never found. It is possible that the positive thermotactic response is intimately related to a function vital for growth, development, or migration. To be screened by the procedure employed, competence in these three processes was mandatory. The diminution but not elimination of the positive response in H0596 and H0813 suggests that there is more than one mutable element

involved in the pathway which controls the positive thermotactic response.

Two other attempts have been made to dissect a thermosensory transduction system with mutation analysis. The nematode, Caenorhabditis elegans, also demonstrates positive and negative thermotaxis (51). With mutants, Hedgecock and Russell were able to separate the positive and negative thermotactic responses. Escherichia coli is capable of positive thermotaxis (52,53) and there is some evidence that it is also negatively thermotactic (52). Mutants which show a diminished chemotactic response to L-serine express a weakened positive thermotactic response (53). Serine is also a potent inhibitor of thermotaxis in E. coli (53). By competition studies with various chemoeffectors and chemosensory mutants, Maeda and Imae were able to demonstrate that serine and the temperature stimulus interact before the methyl-accepting chemotaxis protein (53). Since it has been shown that the serine receptor and the methyl-accepting protein are the same molecule (54,55,56), it appears that the methyl-accepting chemotaxis protein is the thermal receptor.

It is hoped that further analysis of the mutants described here and others, which are currently being characterized, will allow the dissection of the D. discoideum thermosensory transduction pathways at a molecular level. Based upon the analysis of Maeda and Imae (53), testing the responses of these mutants to other

environmental stimuli may be beneficial. The biochemical alterations which result from these mutations are also important and with the advent of parasexual genetic analysis, gene mapping is also possible. With these promising approaches, it is hoped that an understanding of sensory transduction in a eukaryotic organism like Dictyostelium discoideum is possible.

Summary

Amoebae of Dictyostelium discoideum, strain HL50, were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine, cloned, allowed to form slugs and screened for aberrant positive and negative thermotaxis. Three types of mutants were found. Mutant HO428 exhibits only positive thermotaxis over the entire temperature range (no negative thermotaxis). HO596 and HO813 exhibit weakened positive thermotaxis and normal negative thermotaxis. The weakened positive thermotactic response results in a shift toward warmer temperatures in the transition temperature from negative to positive thermotaxis. Mutant HO209 exhibits weakened positive and negative thermotactic responses and has a transition temperature similar to that of the parental strain (HL50). The two types of mutants represented by HO428, HO596 and HO813 support the model that positive and negative thermotaxis have separate pathways for temperature sensing. The type of mutants which contains HO209 suggests that those two pathways converge at some point before the response.

CHAPTER 4

Effect of Stimulus Strength and Adaptation on the Thermotactic Response of *Dictyostelium discoideum* Pseudoplasmodia

Introduction

Dictyostelium discoideum is a cellular slime mold which, as an amoeba, feeds on the bacteria associated with decaying matter. Upon starvation, the amoebae aggregate and form a multicellular mass called a pseudoplasmodium or slug. This slug migrates as a unit and is capable of phototaxis (2,15), chemotaxis (14), and thermotaxis.

Thermotaxis was first reported by Raper as a directed migration toward the warmer side of a temperature gradient (2). This was confirmed by Bonner et al. who discovered the extreme sensitivity of the response (5). Poff and Skokut also found the response to be very sensitive and showed that lowering the growth temperature shifted the range of the response to lower temperatures (16). Later Whitaker and Poff reported that slugs not only migrate toward warmer temperatures (positive thermotaxis), but if the gradient temperatures are cool enough the slugs show directed migration to even cooler temperatures (negative thermotaxis) (17). Whitaker and Poff also reported that by changing the growth and development temperature, the transition from

negative to positive thermotaxis could be shifted; this transition is usually several degrees below the growth and development temperature. Recently, Fisher and Williams have examined the thermotatic properties of D. discoideum slug phototaxis mutants. The mutants, but not the parental strain, exhibited a second thermotactic transition (57). At temperatures above growth temperature these mutants again exhibited negative thermotaxis, but this second transition was not strongly dependent on growth temperature.

Whitaker and Poff have suggested that there are least three sensors which control thermotaxis (17). One sensor was thought to control the positive thermotactic response, another sensor the negative response and the third sensor was thought to mediate adaptation. The strongest evidence in favor of such a hypothesis was the dependence of the lower transition temperature on the strength of the temperature gradient (17); this was based on two gradient strengths. Schneider et al. have isolated D. discoideum thermotactic mutants and found that the positive and negative response are genetically separable (50, Chapter 3). This evidence supports the concept of two pathways, if not separate sensors, for negative and positive thermotaxis. In this chapter, I report the effect of gradient strength and growth/development temperature on the parental strain and on some of these thermotactic mutants.

Materials and Methods

The materials and methods used for the experiments described in this chapter are stated in Chapter 1. The generation and isolation of the D. discoideum thermotaxis mutants has already been described (50, Chapter 3). When it appeared that the saturation of the statistic, r, might be important, the angle which encompassed ninety percent of the slugs was used as a measure of response. This percentage was arbitrarily chosen such that it includes most of the slugs but is not influenced by the few slugs which act contrary to the norm. When this angle was plotted, instead of r, the shape of the resultant temperature-response curves, stimulus-response curves and the apparent saturation points of the responses were unchanged.

Results

The thermotactic response of Dictyostelium discoideum strain HL50 and two mutants, HO428 and HO813, were characterized on 0.11 °C/cm gradients (Figure 7, Chapter 3). Upon comparison with the parental strain, mutant HO428 was found to lack negative thermotaxis while mutant HO813 expressed normal negative but reduced positive thermotaxis. The temperature range within which migration occurred was reduced in both mutants. On a temperature gradient with a midpoint temperature of 28°C, HO428 and HO813 did not migrate. HL50 was able to crawl with a gradient midpoint temperature of 29°C. Mutant HO428 was also not capable of crawling on a gradient with a midpoint temperature of 14°C.

The thermotactic responses of these D. discoideum strains also have been examined on gradients of varying strength (Figures 9,10,11). When strain HL50 was examined on gradients with midpoint temperatures of 16°C and 24°C, the resulting stimulus-response curves were typical of those expected when there is a single response to an environmental stimulus, i.e., the response increased with stimulus strength to a point at which the response appeared to saturate. When the positive thermotactic response of HL50 at 24°C and the negative response at 16°C are compared, the response at 16°C appeared to saturate at a reduced directness and a larger stimulus was needed to induce apparent saturation.

The response of HL50 to thermal gradients of varying strengths, with a midpoint of 18°C, is not typical of a single response. The shape of this stimulus-response curve suggests a minimum of two responses, each with a different dependence on gradient strength. The negative response appears to dominate on weak or shallow gradients, but on gradients with a strength greater than about 0.12 °C/cm the positive response dominates.

When mutant HO428 was examined on gradients of varying strength and midpoint temperatures, a positive thermotactic response was always seen. With midpoint temperatures of 18°C and 24°C, the resultant stimulus-response curves were typical of those of a single response. On the thermal gradients with midpoints of 18°C, saturation seemed to occur

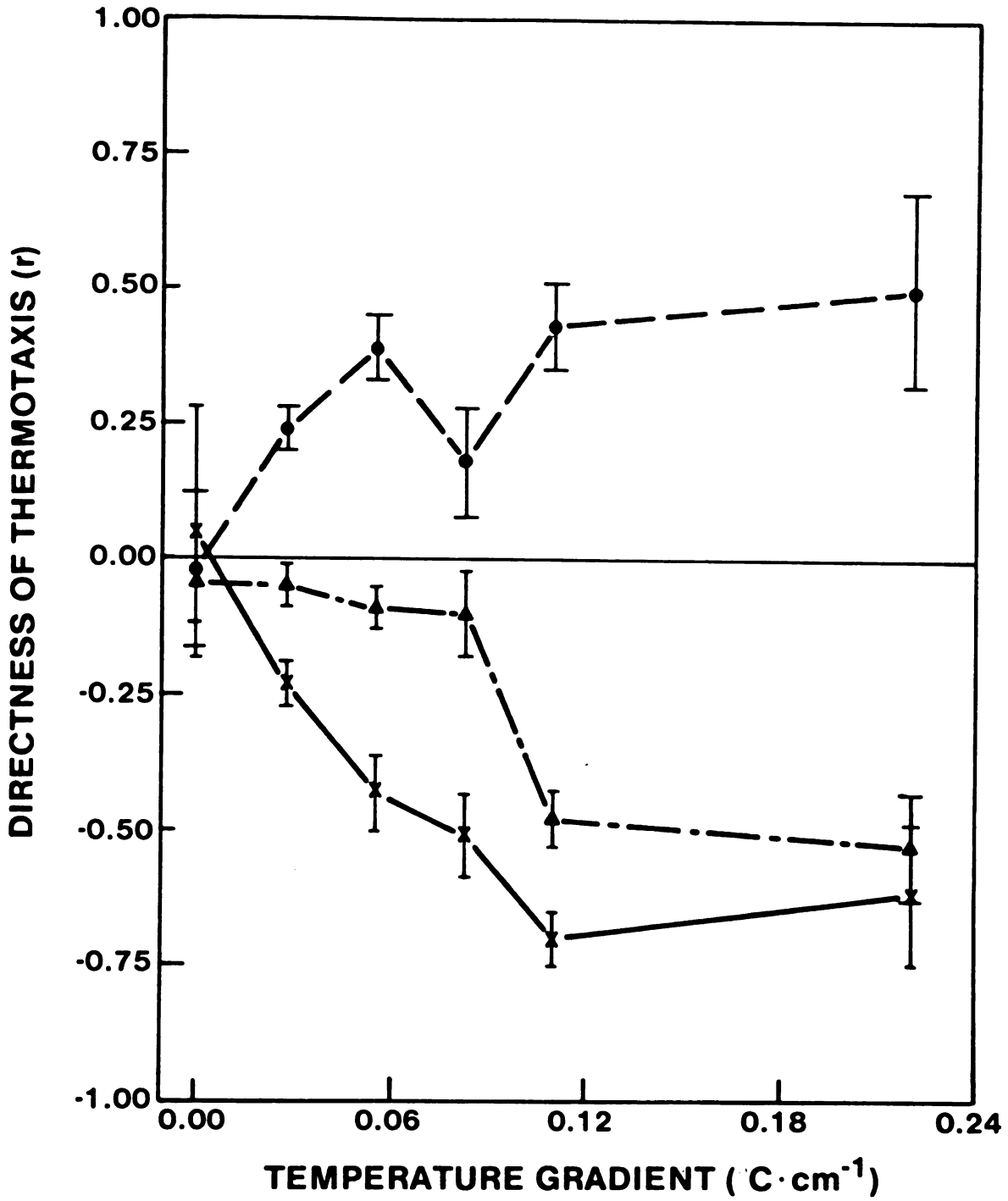

Figure 9. Stimulus-response curves of thermotaxis on gradients with midpoint temperature of 16°C. Each point is the result of at least three separate experiments and the vertical bars represent ± one standard error of the mean. X—X, HL50; ●—● , HO428; ▲—▲ , HO813.

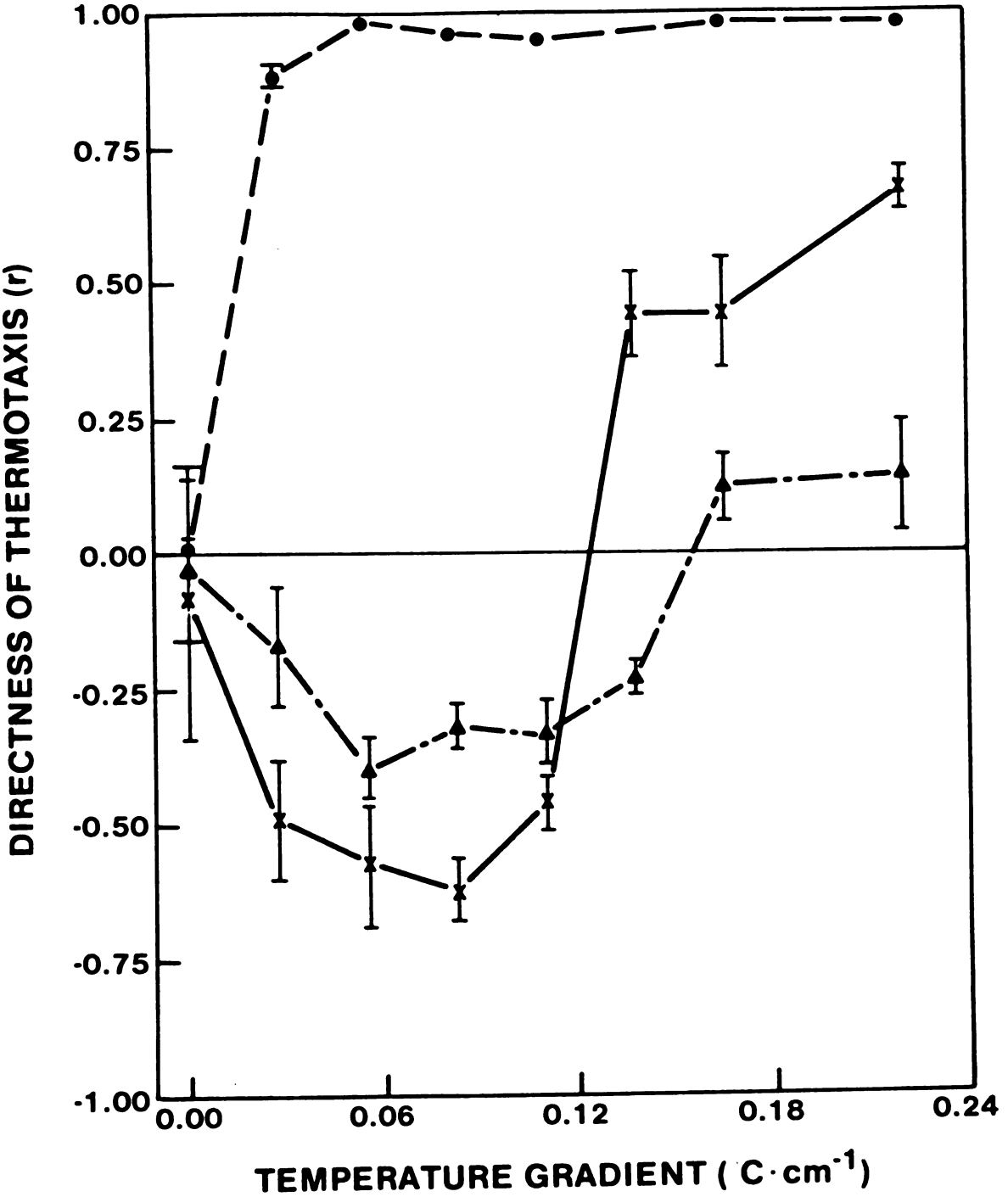

Figure 10. Stimulus-response curves of thermotaxis on gradients with midpoint temperatures of 18°C. Each point is the result of at least three separate experiments and the vertical bars represent ± one standard error of the mean. X—X, HL50; ●—● , HO428; ▲— ▲ , HO813.

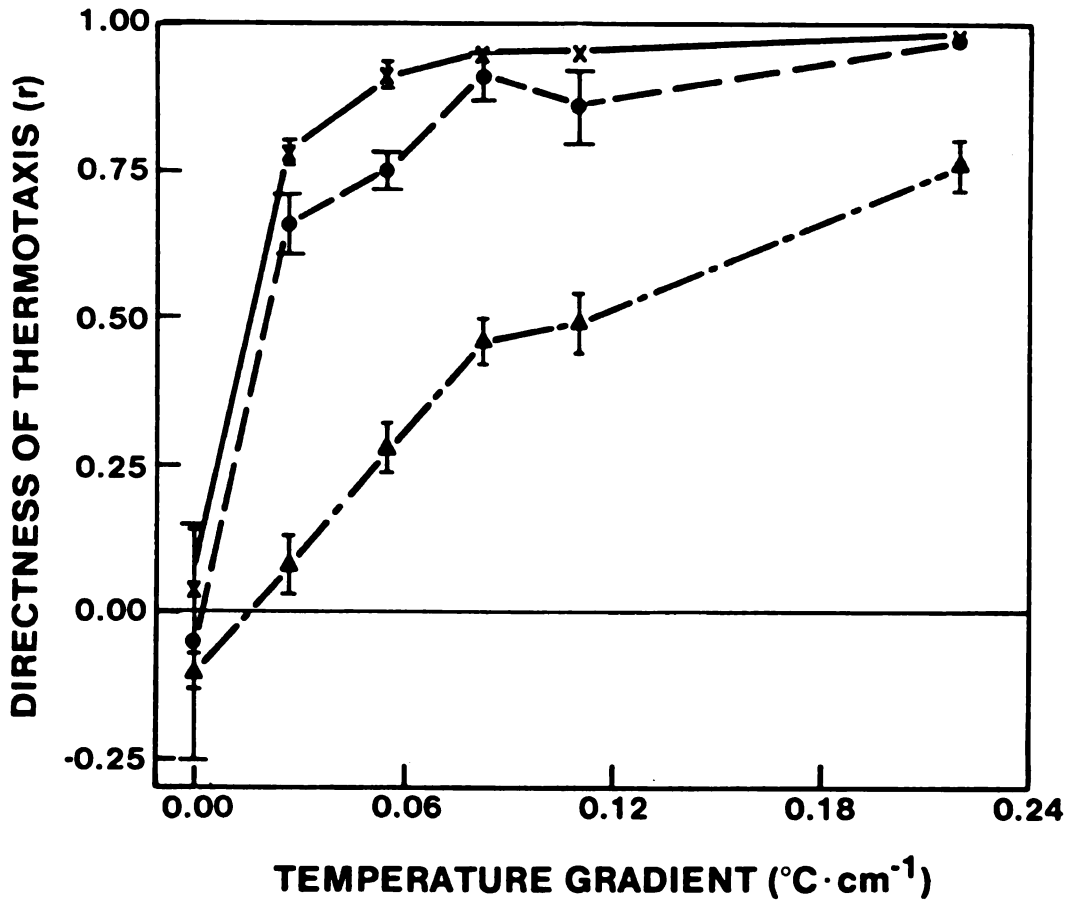

Figure 11. Stimulus-response curves of thermotaxis on gradients with midpoint temperatures of 24°C. Each point is the result of at least three separate experiments and the vertical bars represent ± one standard error of the mean. X—X, HL50; ●—● , HO428; ▲— ▲, HO813.

on a weaker gradient than on those with a 24°C midpoint. Although a positive thermotactic response is always seen, on a 0.22 °C/cm gradient with a midpoint temperature of 16°C, there was evidence of a negative response. On this gradient, a group of slugs migrated toward the cooler temperature. These slugs usually developed into fruiting bodies before crawling more than a couple of centimeters. The other slugs on the plate, the majority, crawled directly toward the warmer temperature and showed little tendency to develop further. Recloning this mutant did not alter this pattern.

Examination of mutant HO813 revealed that, in addition to its weakened positive thermotactic response, its negative response was also weakened. This was most obvious on shallow gradients with a midpoint temperature of 16°C (Figure 9). However, it does appear as if the positive thermotactic response of HO813 was weakened to a greater extent than the negative response. The transition from negative to positive thermotaxis in D. discoideum can be stimulus-induced or induced by changing the gradient midpoint temperature. When comparing these transitions in HL50 and HO813, the stimulus-induced negative thermotaxis to positive thermotaxis transition of HO813 occurred at a greater stimulus strength than that required for the transition in HL50 (Figure 10). The transition, which appeared as the gradient midpoint temperature was varied,

occurred at a higher midpoint temperature with HO813 than with HL50 (Figure 7).

The adaptation of HL50 and HO428 to changes in ambient temperatures was also investigated. The data of Whitaker and Poff suggested that adaptation involved shifts of the transition point and not necessarily the strengthening or weakening of the component responses (17). If mutant HO428 does express the positive response without the negative response, a study of the adaptation of this mutant and HL50 may be valuable in an attempt to understand the adaptation phenomenon in D. discoideum.

In order to examine adaptation, a temperature-response curve was generated with HL50 and HO428 on a 0.03 °C/cm gradient (Figure 12A). These strains were grown and allowed to develop at 23.5°C. If HL50 and HO428 were grown at 27.5°C and allowed to develop at this temperature, there was a substantial change in the resultant temperature-response curves (Figure 12B). All the thermotactic responses were diminished. The apparent negative response was eliminated and the positive response was weakened. The temperature dependence of the positive thermotactic response of HO428 was also shifted, with the maximum closer to the new growth and development temperature, i.e., a maximum at 18°C with growth and development at 23.5°C and a maximum of 22°C with growth and development at 27.5°C. Mutant HO428 exhibited a stronger positive thermotactic response than HL50.

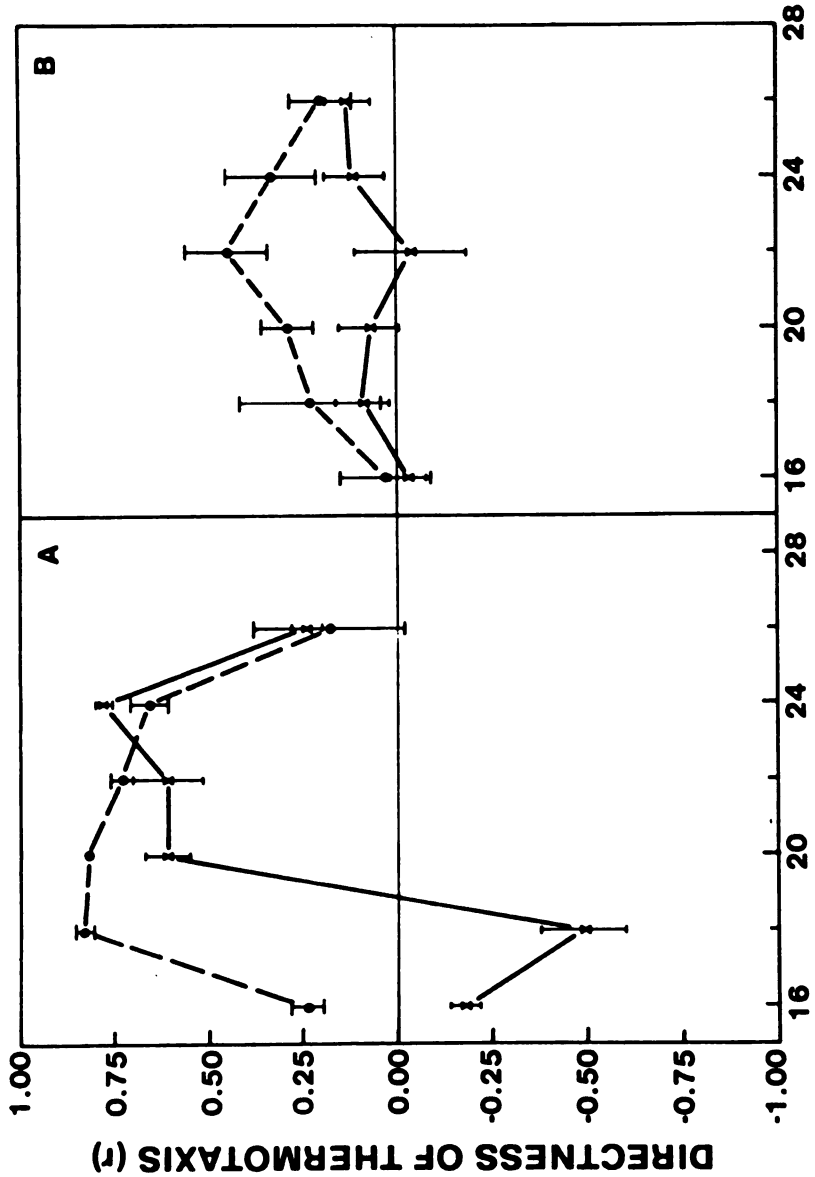

Figure 12. Temperature-response curves of thermotaxis by HL50 and HO428 grown and developed at (A) 23.5°C and (B) 27.5°C. The gradient strength was 0.03 °C/cm. Each point is the average of at least three separate experiments and the vertical bars represent ± one standard error of the mean. X—X, HL50; ●—●, HO428.

If HL50 is grown and allowed to develop at 27.5°C and then placed on a 0.11 °C/cm gradient, thermotactic responses are evident (Figure 13). This suggests that the thermotactic responses are not eliminated by growth and development at this higher temperature, but are reduced in sensitivity as expressed on shallow gradients. These adaptation experiments indicate that raising the temperature of growth and development shifts the temperature at which the positive thermotactic response is maximal and alters the dependences of both thermotactic responses on stimulus strength.

Discussion

It is known that on organism may change the sign of its response as the strength of an environmental stimulus is changed. This phenomenon is most often observed in light-induced responses. For a review see reference (58). In cases where it has been studied further, the change in sign has been indicative of two separable responses (24,58,59,60). These responses may or may not share a receptor.

The sign reversal of a thermotactic response has been reported in D. discoideum (17,57) and in the nematode, Caenorhabditis elegans (51). These reversals were dependent on the gradent midpoint temperature, though results of Whitaker and Poff suggested that the sign reversal in D. discoideum was also dependent on stimulus strength (17,18). The results presented here confirm the dependence of the sign of the response on the strength of the stimulus and

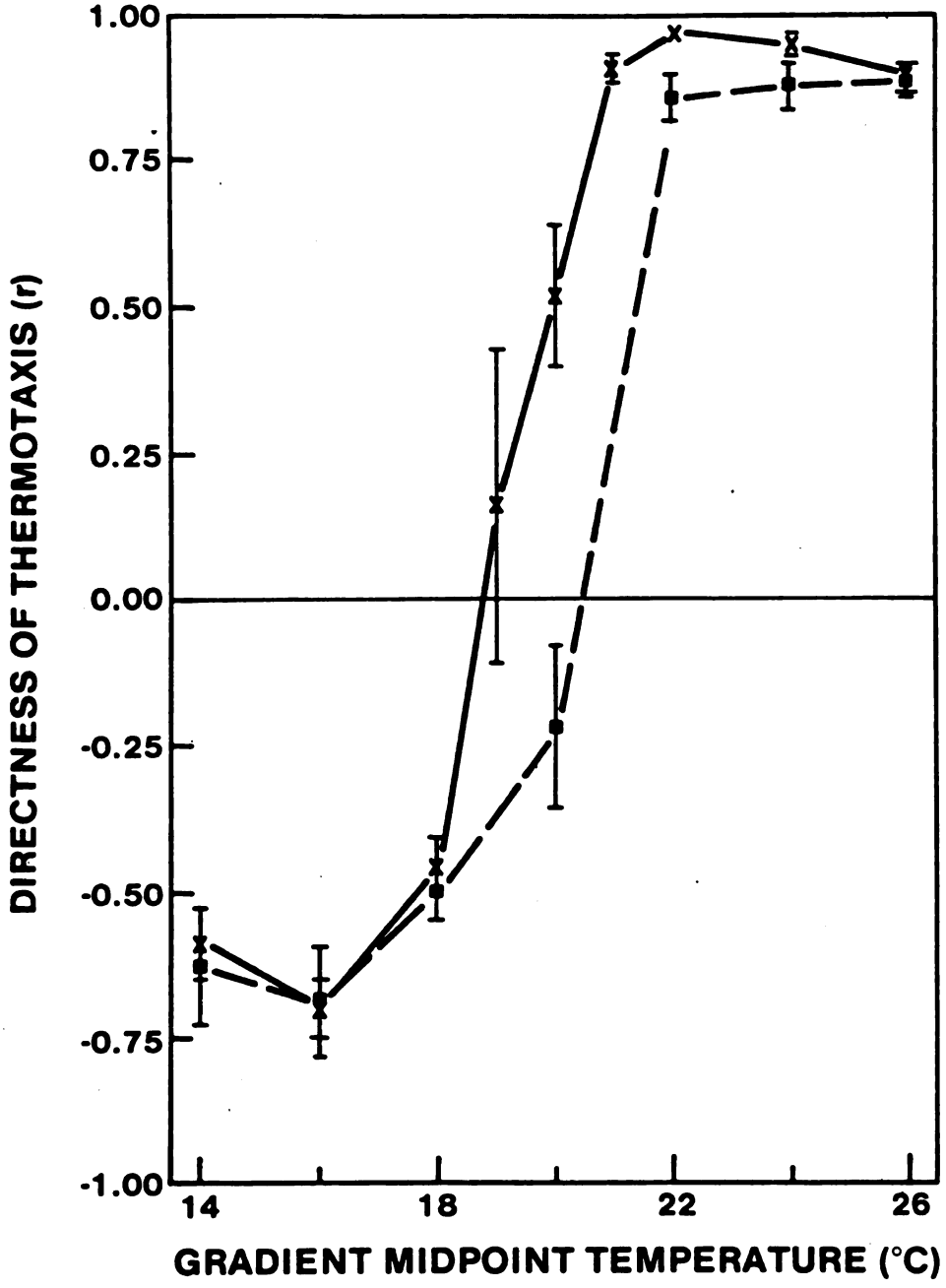

Figure 13. Temperature-response curves of thermotaxis by HL50 on 0.11 °C/cm gradients. Each point is the average of at least three separate experiments and the vertical bars represent ± one standard error of the mean. X—X, HL50 grown and developed at 23.5°C; ■-■, HL50 grown and developed at 27.5°C.

thereby strongly support the conclusion that D. discoideum thermotaxis involves separable response pathways. A similar conclusion was reached, via mutant analysis, for C. elegans (51).

The question arises as to whether the two response pathways are acting concurrently or whether there is a temperature-dependent switch from one response pathway to the other. Results with mutant HO428 suggest that the positive response acts at least in the 16°C to 26°C temperature range. The parental strain, HL50, exhibits a negative thermotactic response at 14°C, 16°C and, with a weak stimulus, at 18°C. The overlap in these temperature ranges suggest that the responses are acting concurrently.

If the responses are acting concurrently, the transition points represent a shift in dominance from one response to the other. The stimulus-induced sign change in HO813 occurs at a larger stimulus strength than that of HL50. This fact, and the observation that HO813 shows a more reduced positive response than negative, are consistent with the conclusion that the two responses act concurrently. Also consistent with this conclusion is the shift in the transition toward higher temperatures that is seen when the temperature-response curve of HO813 is compared to that of HL50.

Comparison of the temperature-response curves of HL50 and HO428 indicates that the negative response is strongest at lower midpoint temperatures and is not evident at

midpoint temperatures above 21°C. The mutants described here did not show the second, high temperature transition reported by Fisher and Williams (57). This could be because of the limited temperature range in which HO428 and HO813 are able to crawl. HO428 may not be expected to show the transition because of its very weak negative response. The difference might also be the result of differences in experimental procedure. Fisher and Williams allowed their amoebae to form slugs on the thermal gradients. The experiments reported here dealt with slugs formed in a temperature-controlled chamber and then placed on the gradient and allowed to respond. This may be an important point because most adaptation occurs during the development of amoebae into slugs (17,18). However, the second transition of Fisher and Williams does suggest that the negative response is still functioning at these higher temperatures (26°-28°C).

The stimulus-response curves of HL50 and HO813 must be interpreted with caution because they are a composite of at least two stimulus-response curves. HO428 expressed mostly one response, the positive one. But when this response is weak, a residual negative response is evident and the resultant stimulus-response curve must also be interpreted with caution. When a stimulus-response curve is a composite, an apparent random or saturated response could be the result of a balance between the two component responses.

The apparent random responses which are expressed by HL50 grown and developed at 27.5°C are an example of this problem. With adaptation, C. elegans modifies the strength of its positive response and its negative response remains unchanged (51). Results with mutant HO428 suggest that with adaptation to higher temperatures, the positive response of D. discoideum is modified. The apparent random response of HL50 suggests that the negative response is also altered during adaptation. If HO428 does indeed express only the positive thermotactic response without a significant negative response, then HL50 must have a negative response which is practically equal to the positive response under these conditions. This balance would result in the apparent random response.

A comparison of the responses of HL50, adapted to 23.5°C and 27.5°C, on 0.06 °C/cm and 0.11 °C/cm gradients suggests that adaptation to higher temperatures, while reducing the sensitivity on weak gradients, does not greatly reduce the response on stronger gradients. The shift in the transition to higher temperatures does suggest that growth and development at higher temperatures reduces the strength of the positive response in comparison to the negative. All these observation on adaptation suggest that adaptation in D. discoideum is not as simple as in C. elegans.

The adaptation which takes place during Dictyostelium discoideum amoebal growth may not affect thermotaxis in the same way at that adaptation which occurs during development.

Therefore, response to altered ambient temperature may appear less complicated if adaptation during amoebal growth is studied from the adaption which occurs during development.

The mutant analysis of Schneider et al. (8) and the results presented in this paper strongly support the idea that there are two sensory transduction pathways operating during D. discoideum thermotaxis. The existence of numerous mutants, such as H0813, which show altered positive and negative responses suggests that there are many common elements in the two pathways. Whether these pathways have a common sensor has not been determined. Biochemical analysis of the thermotactic mutants and the processes acting in adaptation will assist in answering this question and helping us gain a better understanding of Dictyostelium discoideum thermotaxis at a molecular level.

Summary

The thermotactic responses of Dictyostelium discoideum strain HL50 and mutants derived from this strain, H0428 and H0813, have been characterized by stimulus-response curves at gradient midpoint temperatures of 16°C, 18°C, and 24°C. With midpoint temperatures of 18°C and 24°C, the stimulus-response curves of H0428 are typical of those of a single positive response. H0428 does show signs of a negative response with a strong stimulus at 16°C. Stimulus-response curves of HL50 and H0813 on gradients with midpoint temperatures of 16°C and 24°C can also be

interpreted on the basis of one thermotactic response. However, on gradients with the intermediate midpoint temperature of 18°C, these strains exhibited a stimulus-dependent change in the sign of the response. On weak temperature gradients, with a midpoint of 18°C, HL50 and H0813 were negatively thermotatic. As the gradients were strengthened, these strains exhibited positive thermotaxis. The gradient strength required to initiate positive thermotaxis was greater in the mutant than in the parental strain. These observations support the hypothesis that there are two concurrent pathways for thermosensory transduction in D. discoideum. One pathway is thought to result in positive thermotaxis and the other in negative thermotaxis. An investigation of the adaptation of thermotaxis indicated that the stimulus-dependence and temperature-dependence of both thermosensory responses was altered by shifting the growth and development temperature.

CHAPTER 5

Relationship Between Phototaxis and Thermotaxis in *Dictyostelium discoideum* Pseudoplasmodia

Introduction

Dictyostelium discoideum is a cellular slime mold which as a pseudoplasmodium or slug is capable of directed migration in response to environmental stimuli. Its phototactic and thermotactic responses were first reported by Raper (12). Subsequently Bonner et al. suggested that phototaxis was just a special case of thermotaxis (5). This suggestion was based on the sensitivity of the thermal response and the apparent indifference to the wavelength of the light used in phototaxis experiments.

Since the suggestion of Bonner et al., several lines of evidence have emerged which indicate that the two responses are indeed separable. Francis was able to measure an action spectrum for the phototactic response which showed wavelength sensitivity (6). Francis also calculated the resultant temperature gradient across a slug if it was assumed that all the impinging light was absorbed on the side of the slug proximal to the light source (6,61). He concluded that the resultant temperature gradient was not

large enough to result in a thermotactic response. Poff and Skokut, by illuminating a portion of the slug with a vertical microbeam, were able to show that slugs turn toward the illuminated side if the light is infrared and away from the illuminated side if the light is in the visible region of the spectrum (16). This turn away from vertical visible light was predicted by the observation that slug phototaxis operates *via* lens effect (6). This difference in response following stimulation with a heat-generating and a visible wavelength again suggests that *D. discoideum* phototaxis and thermotaxis are separable responses.

Recent advances in the study of sensory transduction have been achieved with mutant analysis. Two early attempts were made to use mutants to probe the relationship between slug phototaxis and thermotaxis. Poff and Skokut reported that HL25, a phototactic mutant, demonstrated normal thermotaxis (16). Mutant P73, another phototactic mutant, was also reported to be normally thermotactic (62). However, these reports emerged before negative thermotaxis (moving toward the cooler rather than the warmer temperature) was observed (17) and before the current, more rigorous, methods of quantitation were applied to the sensory responses of slugs (Chapter 1). Mutant P73 and HL25 also showed a reduced ability to crawl and this may have affected their apparent responses.

Recently Fisher and Williams have isolated mutants of *D. discoidium* with altered slug phototaxis (15). These

mutants exhibit a bidirectional response, i.e., they migrate at a set angle with respect to the incoming light. The angle between the two paths of migrations is dependent on the amount of stray light (scattered by the medium), the concentration of amoebae when the slugs are forming, a slug repellent call Slug Turning Factor, and the mutation in the sensory system. This bidirectional response can also be seen with the parental strain, X22, under certain conditions.

Schneider et al. have reported the isolation (50) and characterization of D. discoideum thermotactic mutants(50, Chapters 3 and 4). The results obtained with these mutants support the suggestion of Whitaker and Poff that positive and negative thermotaxis originate from two separable transduction pathways (17).

With these mutants, there exists the opportunity for studying any possible relationship between slug phototaxis and thermotaxis. When Fisher and Williams further examined their phototactic mutants, they found that all these mutants exhibited altered thermotaxis (57); the accuracy of the positive thermotactic response was reduced and the transition from negative to positive thermotaxis was shifted toward the growth temperature. From their report, it did not appear that there was a consistent change in the negative thermotactic response of their mutants.

In this chapter, I report on the phototactic responses of the thermotactic mutants recently described (50, Chapters

3 and 4). The results suggest that there is a relationship between phototaxis and positive thermotaxis, but that the connection is not via a light-generated temperature gradient.

Materials and Methods

The materials and methods needed for the phototaxis experiments have already been described (Chapter 1). In control experiments, an acrylic filter, 6 mm thick, with an optical density of at least 7 at 430 nm (Polycast Technology Corp., Stamford, Conn) was used to block the light from the sources. If the angle which encompasses 90 percent of the slugs is used as a measure of response, the shapes of the curves and the relative responses are unchanged.

Results

The thermotactic mutants of Dictyostelium discoideum, isolated and characterized as previously described (50, Chapters 3 and 4), showed altered positive and/or negative thermotaxis (Figure 14). Mutant H0428 demonstrates positive thermotaxis throughout the termperature range in which it is able to crawl. However with a gradient midpoint temperature of 16°C and a strong thermal gradient (0.22°C/cm), it does exhibit some signs of a negative response (Chapter 4). Mutant H0209 shows aberrant positive and negative thermotaxis. These two responses appear to be altered to the same extent and it was proposed that the mutation may be in a downstream amplification step after the transduction pathways of positive and negative thermotaxis merge (50, Chapter

3). On the 0.11 °C/cm gradient (Figure 14), mutants H0596 and H0813 demonstrate weakened positive thermotactic responses and near normal negative responses. When H0813 was subjected to gradients of differing strength, it became apparent that its negative response is also weakened (Chapter 4). Mutant H01445 appears to have a weakened positive and an altered negative thermotactic response (Appendix A).

To be certain that there were no external thermal gradients influencing slug migration during phototaxis experiments, control experiments were conducted with the light source turned off. Because the light source may also be a source of heat, an optically dense filter was used to block the light so that the light source could remain on but there would be no light to influence migration. In both cases, the slugs migrated in a random manner. I therefore concluded that the light effects described in this chapter are not influenced by a thermal gradient in the room.

The phototactic responses of the parental strain, HL50, and the thermotactic mutants were measured (Figure 15). The curve of fluence rate versus response for HL50 is typical for a photoresponse. At low light intensities, there is no response. At greater light intensities, the response increases linearly when plotted against the log of the intensity. At even greater light intensities the response saturates. The fluence rate versus response curve for mutant H0209 has a similar shape when comparing it to the parental, but the overall response is reduced. This

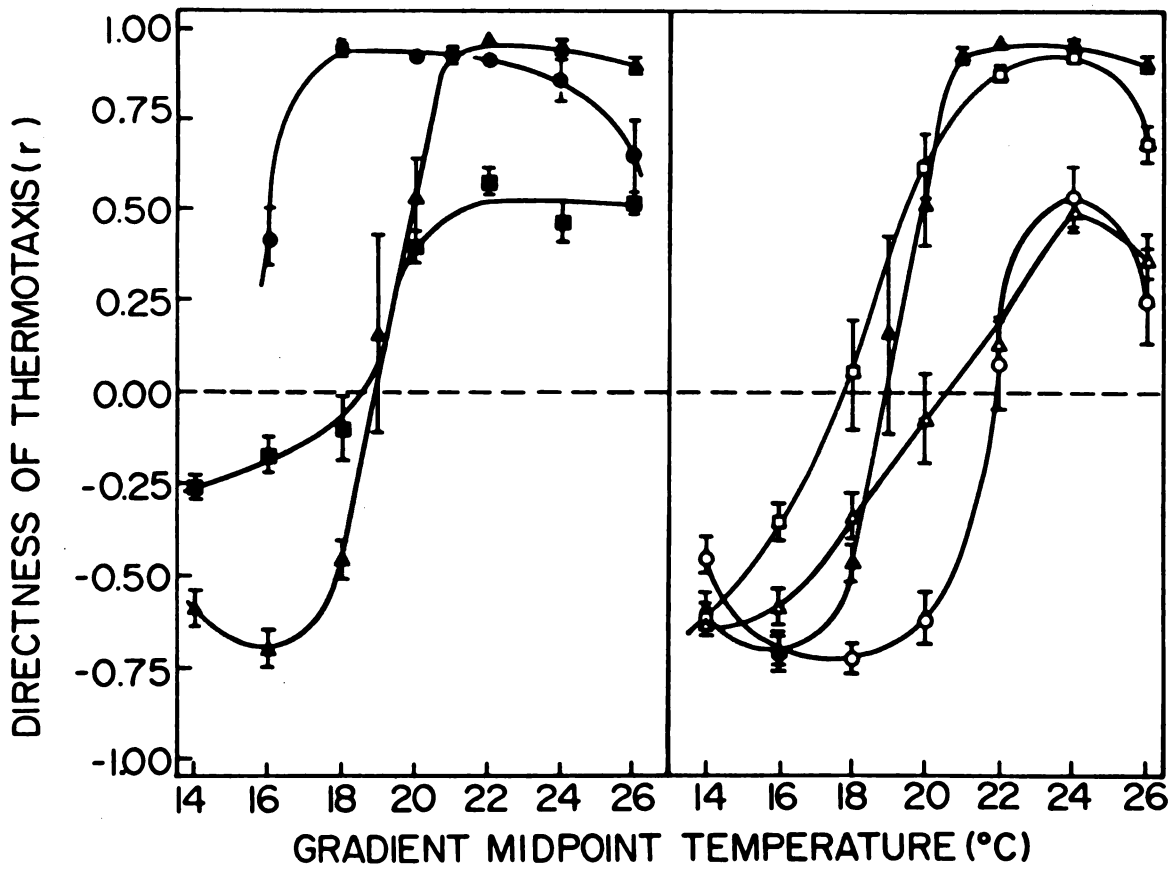

Figure 14. Temperature-response curves of thermotaxis for HL50 and mutants. The temperature gradient was 0.11 °C/cm. At least five separate experiments were used to determine each point and the vertical bars represent ± one standard error of the mean. ▲—▲, HL50; ■—■, H0209; ●—●, H0428; O—O, H0596; Δ—Δ, H0813; □—□, H01445.

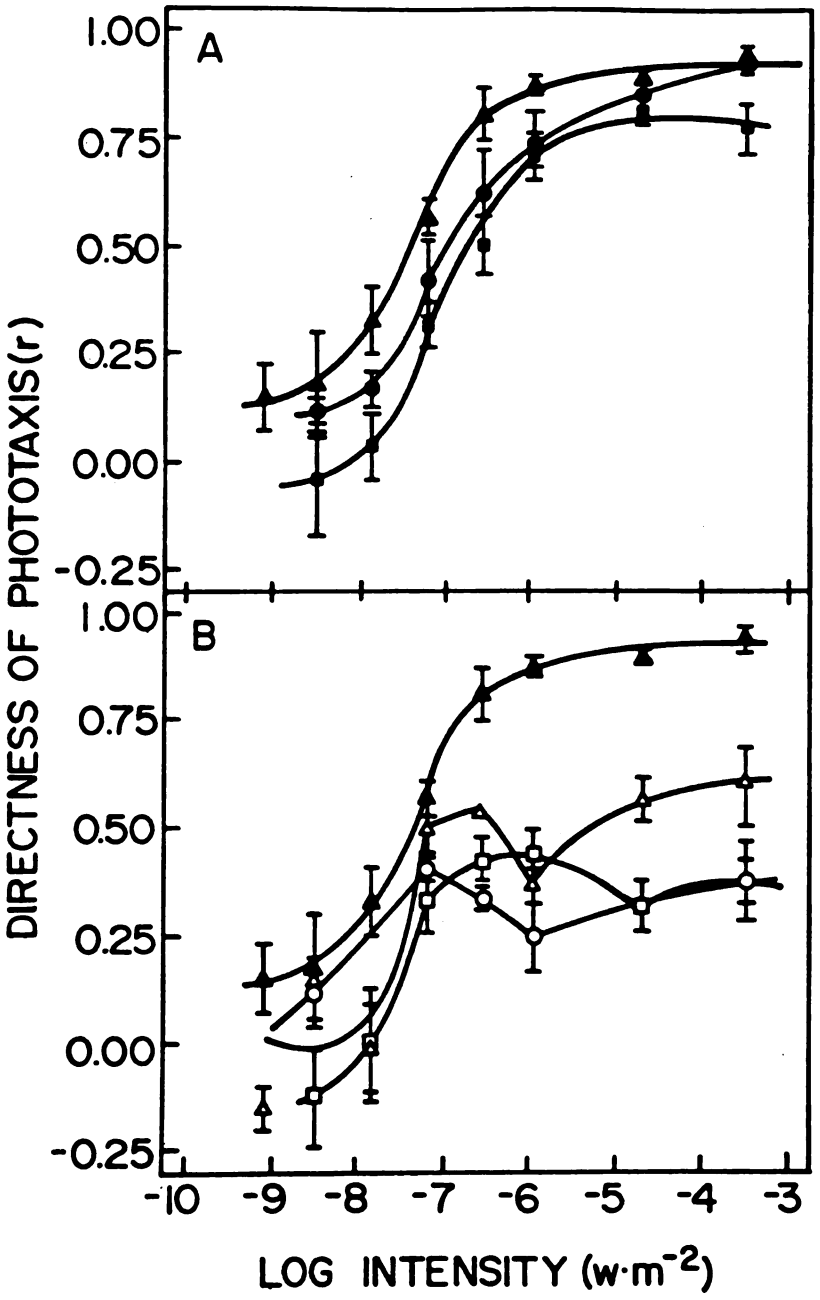

Figure 15. Fluence rate versus response curves of phototaxis for HL50 and mutants. At least three separate experiments were used to determine each point and the vertical bars represent ± one standard error of the mean. ▲—▲, HL50; ■—■, H0209; ●—●, H0428; 0—0 , H0596, Δ—Δ, H0813; □—□, H01445.

observation is consistent with the interpretation of Schneider et al. that this mutation is not in the early steps of transduction but downstream, closer to the response (50). This observation also supports the conclusion of Fisher and Williams that the transduction pathways for phototaxis and thermotaxis converge before the response (57). The phototactic response of mutant HO428 was similar to those of HL50 and HO209, with an intermediate sensitivity to light. This mutant is able to achieve levels of phototaxis comparable to the parental strain, but only at higher light intensities. All of these strains, HL50, HO209, and HO428, demonstrate a phototactic response similar to the responses first reported for D. discoideum (2,5), i.e., the slugs appear to be crawling directly toward the light source.

When the photoresponses of mutants HO596, HO813, and HO1445 were examined, these mutants exhibited seemingly unidirectional phototaxis at light intensities less than those required to saturate the response of HL50. At these low light intensities the response was reduced in magnitude compared to that of the parental strain. At saturating light intensities, these mutants exhibited bidirectional phototaxis (Figure 16) which in turn led to a decrease in the resultant r value. At even higher light intensities, the bidirectionality of the response diminished, i.e., some

Figure 16. Redrawn shadowgraphs of D. discoideum exposed to different intensities of unilateral light. The lines represent the trails of slime sheath left behind by the slugs migrating out from the inoculum spot. a, 6 x 10^{-6} w/m^2; b, 1 x 10^{-4} W/m^2; c, 3 x 10^{-2} W/m^2.

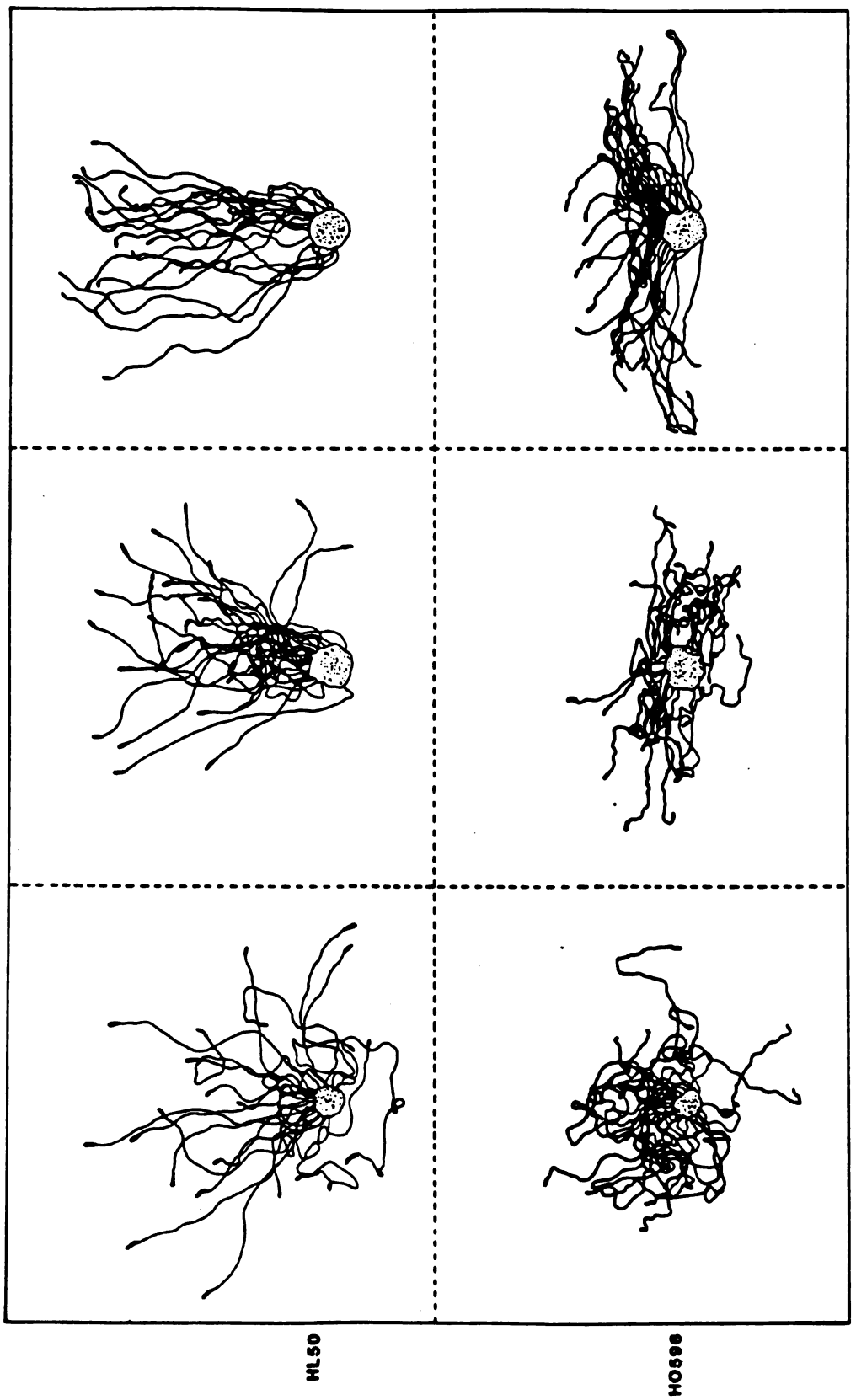

Figure 16

slugs again appeared to be migrating toward the light source. This reduced bidirectinality resulted in an increase in r.

The bidirectional phototactic reponse appeared to be correlated with reduced positive thermotaxis (Figures 14,15). Mutant H0596 and H0813 show a definite reduction in their positive thermotactic response and at intermediate light intensitites their photoresponse is strongly bidirectional. Mutant H01445 exhibits a slightly reduced positive thermotactic response and also exhibits bidirectional phototaxis. Mutant H0428 does not show an apparent bidirectional phototactic response at any light intensity. H0428 does show a thermotactic response similar to H01445 at gradient midpoint temperatures of 22°C to 26°C (Figure 14). However, the positive thermotactic response of H0428 is stronger than that of mutant H01445 when the gradient strength is reduced (see Appendix A, Figure 11). The phototactic responses of these mutants defective in thermosensing indicate a relationship between positive thermotaxis and phototaxis. No similar relationship appears to exist between phototaxis and negative thermotaxis. Mutant H0209 exhibits apparent unidirectional phototaxis. Because it appears that H0209 is defective in a step late in the thermosensory transduction pathway, this unidirectional phototactic response suggests that the interaction between phototaxis and positive thermotaxis occurs before the mutated step.

The previous results suggest that the phototactic response may be dependent on ambient temperature as is the positive thermotactic response. To test this, a fluence rate versus response curve was generated, with strains HL50 and HO813, at an ambient temperature of 16°C instead of 23.5°C (Figure 17). Examination of these curves reveals that a change in ambient temperature does not alter the threshold light intensity, i.e., that intensity necessary to evoke a phototactic response. With HL50, the switch to 16°C increased the magnitude of the response at all but the highest light intensity tested. This was also evident when the angle which encompassed 90% of the slugs was used as a measure of response. With mutant HO813, lowering the ambient temperature decreased the bidirectionality of the response (Figure 18). The resultant fluence rate versus response curve has a shape similar to that of HL50, the parental strain.

To examine further the effect of temperature on phototaxis, phototaxis was measured as a function of temperature. The light intensity at which the response had previously shown the greatest dependence on temperature was chosen. The phototactic response does vary with ambient temperature, and the dependence on ambient temperature is similar for HL50 and HO813 (Figure 19). At temperatures of 18°C and above, these temperature dependence curves are similar to the thermotactic temperature response curve for mutant HO428. At temperatures lower than 18°C, the

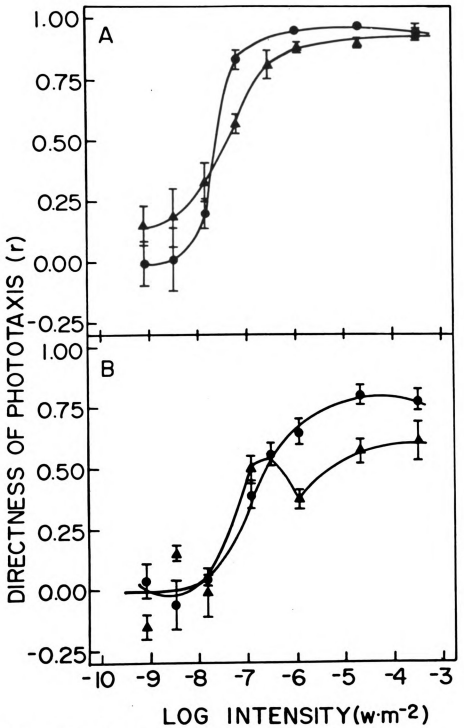

Figure 17. Fluence rate versus response curves of phototaxis for (A) HL50 and (B) HO813. ●—● , 16°C; ▲—▲, 23.5°C. At least three separate experiments were used to determine each point and the vertical bars represent ± one standard error of the mean.

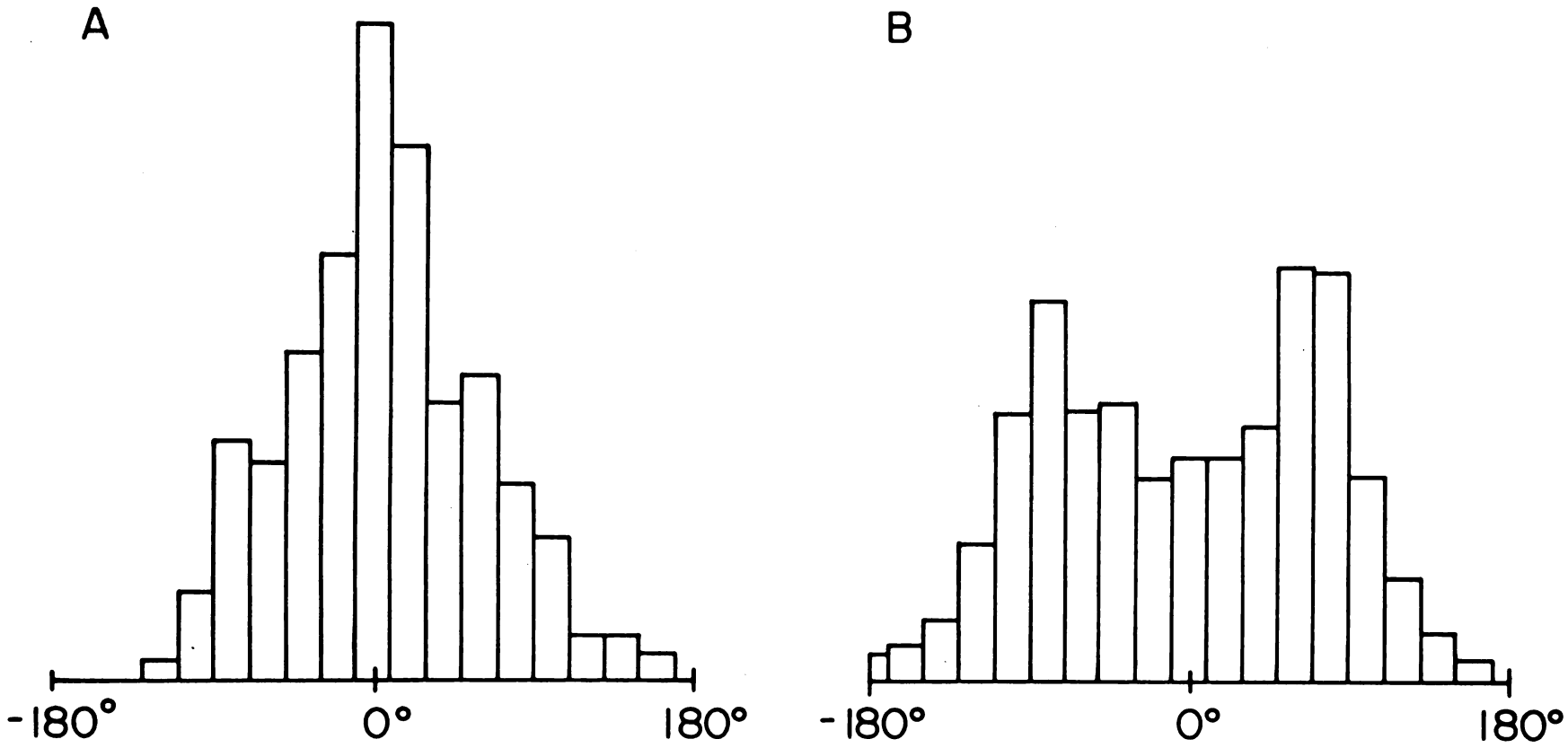

Figure 18. Relative angular distribution of HO813 after phototaxis at (A) 16°C and (B) 23.5°C. The light was placed at 0° and had an intensity of 1 x 10^{-6} W/m^2.

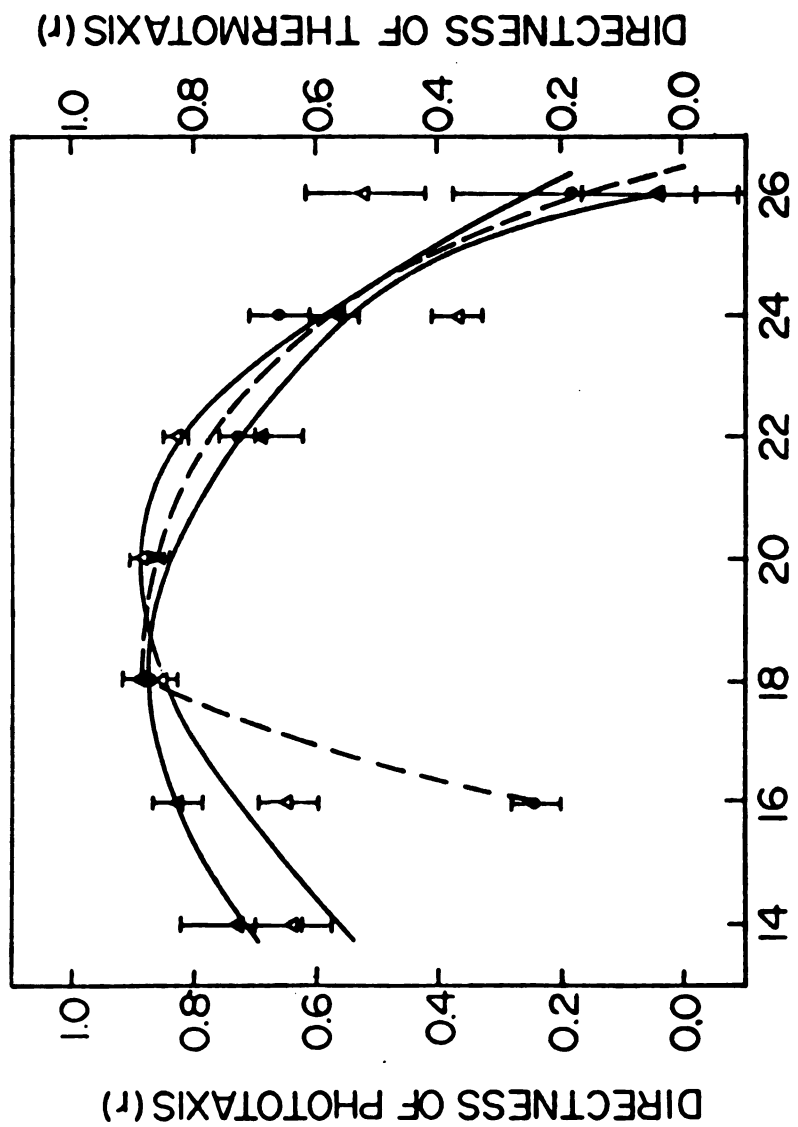

Figure 19. Temperature-response curves of phototaxis (▲—▲, HL50; △—△, HO813) and thermotaxis (●—●, HO428). The light intensity used for HL50 was 6×10^{-8} W/m^2 and for HO813 was 1×10^{-6} W/m^2. The thermal gradient was 0.03 °C/cm. At least separate experiments were used to determine each point and the vertical bars represent ± one standard error of the mean.

thermotactic response of HO428 appears to diminish. This apparent diminution in the positive thermotactic response may be the result of a contribution from the negative response which can be seen at this temperature on temperature gradients of greater strength. Because the response of mutant HO428 is the best approximation available for the positive thermotactic response, the similarity between its thermotactic temperature response curve and the phototactic temperature response curves for HL50 and HO813 is significant.

Discussion

The results of Fisher and Williams (57) and those just presented indicte that bidirectional phototaxis is seen in strains of D. discoideum which have a weakened positive thermotactic response. In addition, Fisher and Williams were able to accentuate the bidirectionality by subjecting the slugs to conditions which would weaken a phototactic and possible thermotactic response (15). We show in this paper that bidirectionality also depends on light intensity and ambient temperature, factors which also affect phototactic and thermotactic responses.

The relationship between bidirectionality and positive thermotaxis suggests that the bidirectional response demonstrated by mutant HO813 may be the parental response if a concurrent thermotactic response is weakened. Closer examination reveals that this idea is not correct. At 16°C, strains HL50 and HO813 should respond to a light-generated

temperature gradient with a negative thermotactic response. This negative response would reduce the apparent magnitude of the phototactic response by increasing the tendency of the slugs to crawl away from the light source. Under these conditions, the bidirectionality of the response of HO813 should have been increased. However, lowering the ambient temperature increased the phototactic response of HL50 and almost eliminated the bidirectionality of the HO813 response. This indicates that the relationship between phototaxis and thermotaxis is not via a light-generated temperature gradient.

At 23.5°C and at light intensities above 10^{-6} W/m^2, slugs of strains HO596 and HO813 show reduced bidirectionality in their phototactic response. Again the question of a possible light-generated temperature gradient arises. The minimum temperature gradient that HO813 will respond to at this ambient termperature is around 0.03°C/cm (Chapter 4). The light intensity needed to generate this gradient, calculated by the method described by Francis (6) and Gamble (61), is around 2 W/m^2. This calculation indicates that a light-generated temperature gradient is probably not involved in any of the effects described in this paper. However, this explanation can not totally be eliminated when considering the decrease in bidirectionality because an amplification system within the slugs is possible. An amplification system, such as a light-induced uncoupling of respiration, could increase any

light-generated temperature gradient. Amoebal phototaxis might also explain the decrease in bidirectionality observed at higher light intensities.

The data presented in the chapter suggest that D. discoideum slug phototaxis and positive thermotaxis are related, but not via a light-generated temperature gradient. The similarity between the phototactic temperature versus response curves and the thermotactic temperature versus response curve of strain HO428 suggests that the relationship may be through a common step or steps in the pathway. This relationship between two sensory systems is not unique to D. discoideum. The thermotactic, phototactic, and chemotactic responses of Escherichia coli appear to be interrelated (52,53,63). A relationship between thermotaxis and chemotaxis has been observed in other eukaryotic organisms (51,64).

Whitaker and Poff have postulated that there is a sensor in D. discoideum which detects absolute temperature and controls adaptation to a changing ambient temperature (17). The results presented here strongly suggest that D. discoideum does have such a temperature sensor and that this sensor is capable of modulating the phototactic response.

Summary

The phototactic response of Dictyostelium discoideum thermotactic mutants was investigated. Mutants which demonstrated reduced positive thermotaxis exhibited bidirectional phototaxis, but only at light intensities

which saturated the response of the parental strain. No correlation could be drawn between negative thermotaxis and phototaxis. The accuracy of the phototactic response of the parental strain and the degree of bidirectionality in the response of the mutants was dependent on ambient temperature. The threshold intensity for phototaxis was temperature independent. The temperature dependence of phototaxis of strains HL50 and HO813 was similar to the that of thermotaxis exhibited by a strain which only shows a positive thermotactic response. This suggests a relationship between phototaxis and positive thermotaxis but this relationship is not via a light-generated temperature gradient.

CONCLUSION

As a result of their studies on Dictyostelium discoideum strain NC-4, Whitaker and Poff proposed that there are three sensors involved in D. discoideum slug thermotaxis; one sensor is thought to control the positive response, another the negative response and the third to regulate adaptation (17,18). It was further proposed that the sensor which mediates adaptation is a Δ-5 fatty acid desaturase, whose activity is capable of altering the fluidity of the membranes (17,21). In this dissertation, I have examined these proposals as well as the suggestion by Bonner et al. that phototaxis is just a special case of thermotaxis (5).

Shortly after it was suggested that a fatty acid desaturase might regulate adaptation, Mohan Das et al. reported that changing the growth temperature did not produce systematic alterations in the fatty acids of amoebae of D. discoideum strain AX-2, and that the change in growth temperature had no effect on membrane fluidity as determined by ESR (43). If strain AX-2 is capable of 'wild type' thermotactic responses, these results are contrary to those predicted by the Whitaker and Poff hypothesis. Therefore, the thermotactic responses of strain AX-2 were examined and

AX-2 was found to demonstrate positive and negative thermotaxis. These responses were also dependent on the growth and development temperature, as were the responses of strain NC-4. The fluidity of the lipids from strain AX-2 was examined after the D. discoideum was allowed to develop into slugs at 18°C or 23.5°C and the fluidity was found to be independent of development temperature. These results, along with those of Mohan Das et al. (43), suggest that bulk lipid fluidity is not altered during adaptation, and that a fatty acid desaturase is probably not the temperature sensor which regulates adaptation.

To determine if positive and negative thermotaxis are mediated by separate sensors, mutants which demonstrated aberrant thermotaxis were examined. A mutant was found, HO428, which had a normal positive thermotactic response, but never displayed negative thermotaxis. Another mutant, HO596, appeared to have a normal negative thermotactic response and a weakened positive response. Moreover, two mutants were found which had an alteration in both responses; HO209 had its positive and negative thermotactic responses altered by approximately equal amounts and HO813 showed a greater diminution of its positive response than its negative. The responses of these mutants indicate that positive and negative thermotaxis have genetically separable pathways as well as common elements.

When the thermotactic response of the parental strain, HL50, and mutants HO428 and HO813 exhibited a

stimulus-dependent change in the sign of the response. With a midpoint of 18°C at low gradient strength, HL50 and HO813 demonstrated negative thermotaxis. As the strength of the gradient was increased, HL50 and HO813 displayed positive thermotaxis. The gradient strength necessary to induce the positive response was greater for the mutant than for the parental strain. These results, along with the temperature-response curves for these strains and strain HO428, strongly support the conclusion that positive and negative thermotaxis are the result of separable pathways. The data also indicate that the two pathways act concurrently and that the response which is strongest on that particular gradient determines the direction of slug migration. Whether the positive and negative thermotactic responses share the same sensor can not be determined from the results presented here and an understanding of the pathways on a molecular level will probably be necessary before this question can be answered definitively.

To study further the possible relationship between slug thermotaxis and phototaxis, the phototactic response of the parental strain and the thermotactic mutants was investigated. Mutants with a weakened positive thermotactic response exhibited bidirectional phototaxis. No correlation could be found between negative thermotaxis and phototaxis.

To determine whether the phototaxis-thermotaxis relationship was <u>via</u> a light-generated temperature gradient, the temperature dependence of phototaxis was measured. At

temperatures where the slugs would demonstrate negative thermotaxis if exposed to a thermal gradient, the phototactic response was enhanced and in those mutants which display bidirectional phototaxis, the bidirectionality decreased. The temperature-dependence of phototaxis was similar to the temperature-dependence of the thermotactic response of the mutant which displayed only positive thermotaxis, HO428. These results also suggest a relationship between slug phototaxis and thermotaxis, but indicate that they are not related by a light-generated temperature gradient.

RECOMMENDATIONS

Future studies on the thermotactic responses of Dictyostelium discoideum should deal with the genetics and biochemistry of the system. The mutants obtained thus far should be back-crossed so as to obtain strains with just one lesion, the lesion which affects the thermotactic response. When these strains have been obtained, their proteins should be compared to those of the parental strain. It might be helpful to first examine membrane proteins because the initial step(s) in thermotaxis are probably membrane associated.

Another promising area for future resesarch involves adaptation. By studying HL50 and HO428, a better understanding of how adaptation alters each thermotactic response, positive and negative, is possible. The adaptation which occurs during growth should be studied separately from that adaptation which occurs during development. I believe that if the two are studied separately, the alteration in the responses will not appear as complex as suggested in Chapter 4. Once the adaptation response has been characterized phenomenologically, a complete analysis of the proteins and lipids should be carried out and temperature-dependent alterations should be

compared kinetically with alterations in the thermotactic responses. Since methylation and phosphorylation of lipids and proteins are often involved in adaptation responses, a less tedious, but still promising approach might be to search for these. This can be done by indubating with radioactive compounds, before the adaptation period, and examining the subsequent incorporation patterns.

APPENDICES

APPENDIX A

Characterization of *Dictyostelium discoideum* strains HO1395 and HO1445

After the first 1000 mutagenized clones were screened for thermotaxis and phototaxis mutants, Dr. Michael Schneider left the laboratory and subsequent mutagenesis/screening was performed by Dr. Ken Poff, Choo Hong and Douglas DeGaetano. Unfortunately, with the switch in personnel, the kill rate rose and the incidence of mutants in thermotaxis and phototaxis also increased. In the first 1000 mutagenized clones screened, six demonstrated aberrant thermotaxis or phototaxis. In the next 500 clones screened, 25 thermotactic or photactic mutants were found. At one point during the screening of this second group, 18 mutants were found when 118 clones were screened. These 118 clones were from the same mutagenesis. Considering the increase in kill rate and the increase in the incidence of thermotactic and phototactic mutants, it can not be assumed that the 25 mutants generated by Poff, Hong and DeGaetano have only one defect in the section of the genome which controls sensory transduction.

Among these last 25 mutants, 2 were reported to show bidirectional phototaxis and unimpaired positive

thermotaxis. These mutants were HO1395 and HO1445 and were 2 of the 18 mutants found among 118 clones. Because these mutants appeared to disprove the conclusion stated in Chapter 5, that bidirectional phototaxis is linked to weakened positive thermotaxis, these mutants were examined further.

The thermotactic responses of these mutants were characterized (Figures 20,21,22). Comparing the thermotactic responses of HL50 and HO1395, the responses are similar except on 0.11 °C/cm gradients with midpoint temperatures of 18°C and 20°C (Figure 20). One the 18°C gradient, HO1395 exhibits a positive thermotactic response and HL50 shows a negative response. This shift in the transition point implies that either the negative thermotactic response of HO1395 is weakened at 18°C and 20°C or that the positive response is strengthened at these temperatures. The stimulus-response curves with midpoints at 16°C and 24°C suggest that the strengthening or weakening is expressed only on those gradients with the intermediate midpoints of 18°C or 20°C (Figures 21,22), and is not a general alteration in either the positive or negative response.

When the phototactic response of HO1395 was examined, bidirectional phototaxis was not evident. The photoresponse of HO1395 was comparable to that of the parental strain (Figure 23). Because this mutant does not exhibit bidirectional phototaxis, its behavior does not contradict

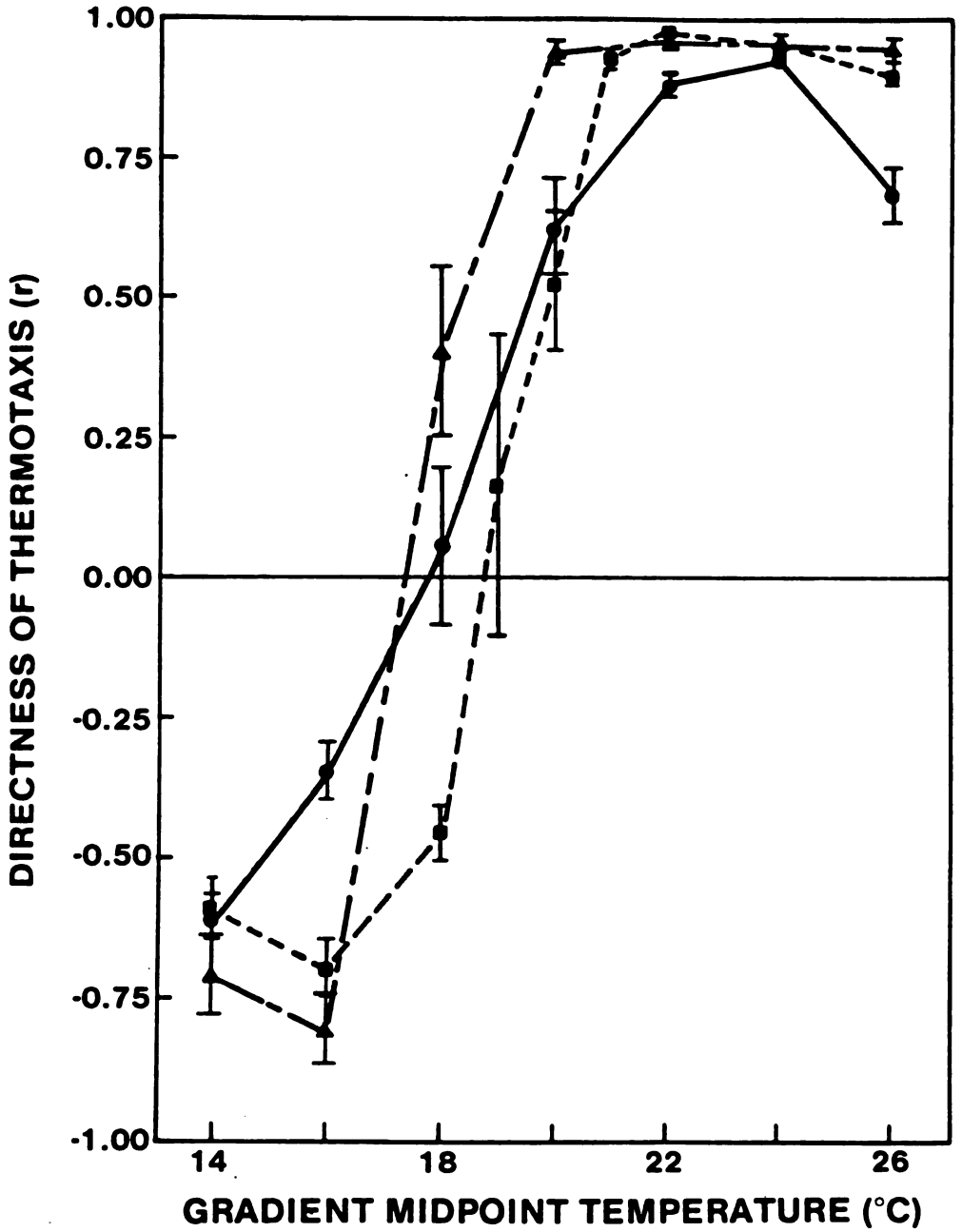

Figure 20. Temperature-response curves of thermotaxis of HL50 and mutants of thermotaxis. The gradient strength was 0.11 °C/cm and the results of at least three separate experiments were used to determine each point. The vertical bars represent ± one standard error of the mean. ■—■, HL50; ▲—▲, HO1395; ●—● , HO1445.

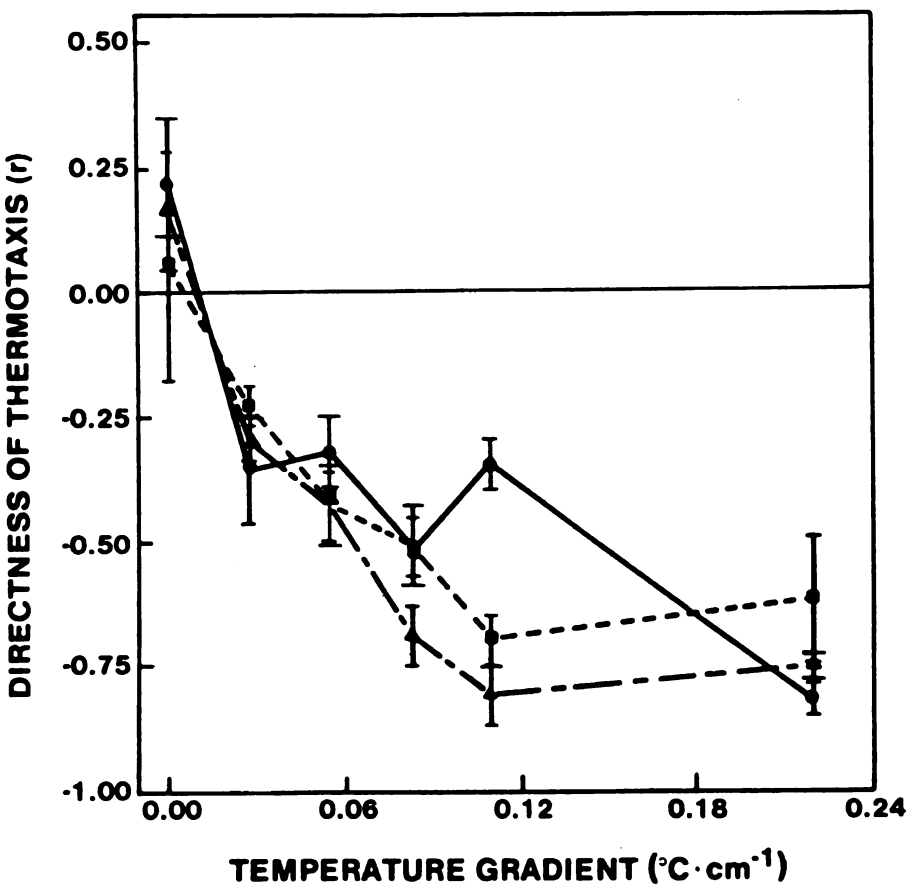

Figure 21. Stimulus-response curves of thermotaxis on gradients with midpoint temperatures of 16°C. Each point is the result of at least three separate experiments and the vertical bars represent ± one standard error of the mean. ■—■, HL50; ▲—▲, HO1395; ●—●, HO1445.

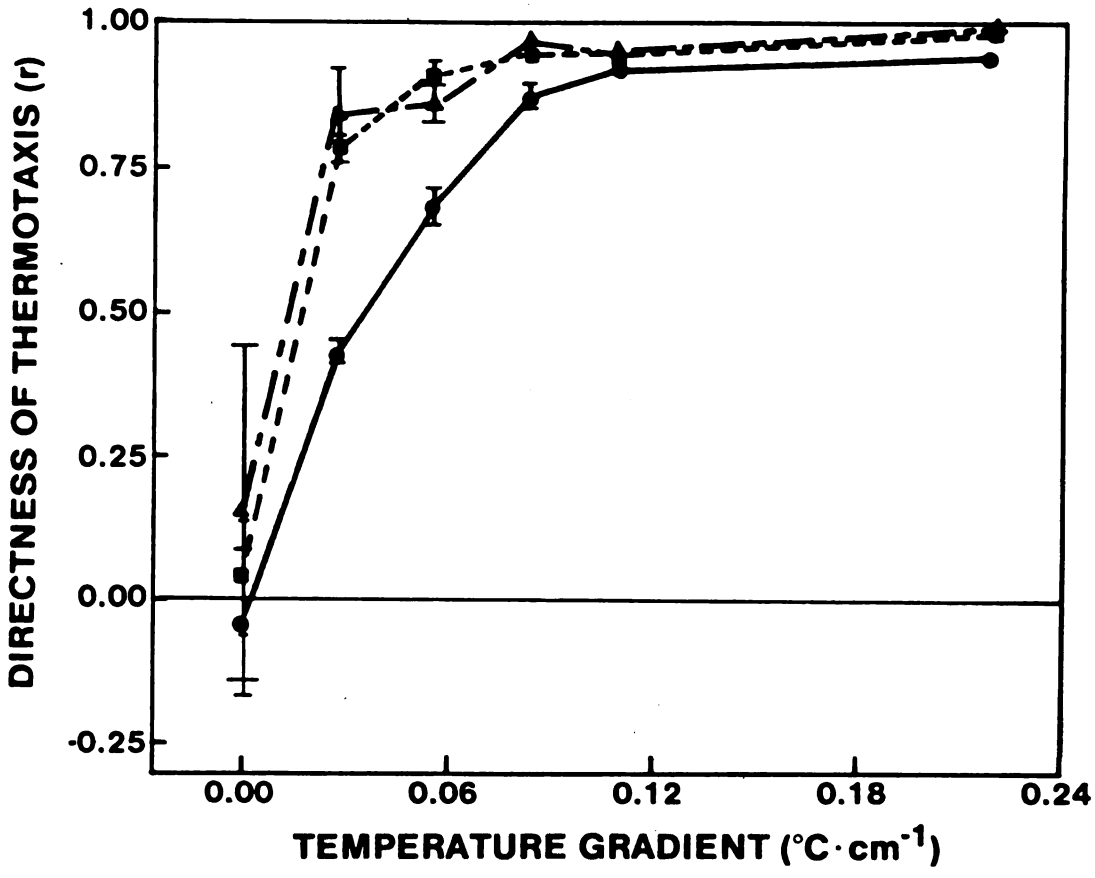

Figure 22. Stimulus-response curves of thermotaxis on gradients with midpoint temperatures of 24°C. Each point is the result of at least three separate experiments and the vertical bars represent ± one standard error of the mean. ■—■, HL50; ▲— ▲, HO1395; ●—● , HO1445.

the observed relationship between positive thermotaxis and bidirectional phototaxis.

Mutant HO1445 has a weakened positive thermotactic response (Figures 20,22) and, with the exception of its behavior on 0.11 °C/cm gradients with midpoints of 16°C and 18°C (Figures 20,21), a near normal negative response. The response of HO1445 on the gradient with the 16°C midpoint was tested several times and this abnormal response appears real. Again, as with HO1395, the transition from negative to positive thermotaxis is shifted to a temperature below 18°C.

When the photoresponse of HO1445 was examined, this mutant did exhibit bidirectional phototaxis (Figure 23). For this reason, it was included in the discussion of this response in Chapter 5. This mutant also exhibits abnormal amoebal phototaxis (C. Hong, personal communication); it does not demonstrate the strong positive response at intensities of white light around 10^{-6} W/m^2.

Both HO1395 and HO1445 have interesting alterations in their sensory responses. However, before too many conclusions can be drawn from the behavior of these mutants, it should be demonstrated that these alterations are the result of a single mutation.

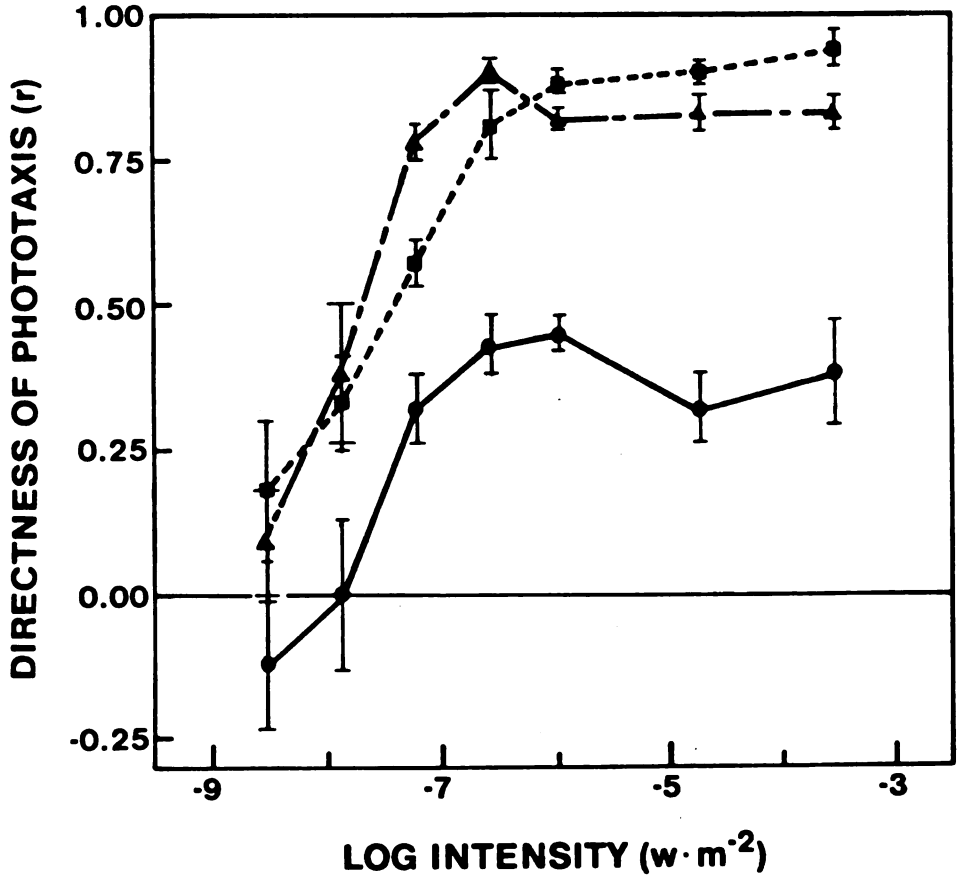

Figure 23. Fluence rate versus response curves of phototaxis of HL50 and mutants. At least three separate experiments were used to determine each point and the vertical bars represent ± one standard error of the mean. ■—■, HL50; ▲—▲, HO1395; ●—●, HO1445.

APPENDIX B

A Model for Bidirectional Phototaxis

The bidirectional phototactic response of some mutants of D. discoideum indicates that in these mutants, the tendency to turn toward the light is balanced by a tendency to turn away from the light. If one defines the tendency to turn toward the light as positive phototaxis, the tendency to turn away from the light is negative phototaxis. The question that arises is whether positive and negative phototaxis can be explained on the basis of one response pathway, or whether two are required as in slug thermotaxis. A model has been proposed by Dr. Ken Poff which explains this positive and negative response without assuming a second transduction pathway. This appendix is a brief statement of that model.

Slug phototaxis operates via a lens effect. The difference in the index of refraction between the air and the body of the slug causes the incoming light to be focused onto the distal side of the slug (Figure 24). The slug then turns away from the side of greater light intensity; it turns toward the light source.

Photoresponses such as those exhibited by the slug increase in magnitude with increasing light intensity, but

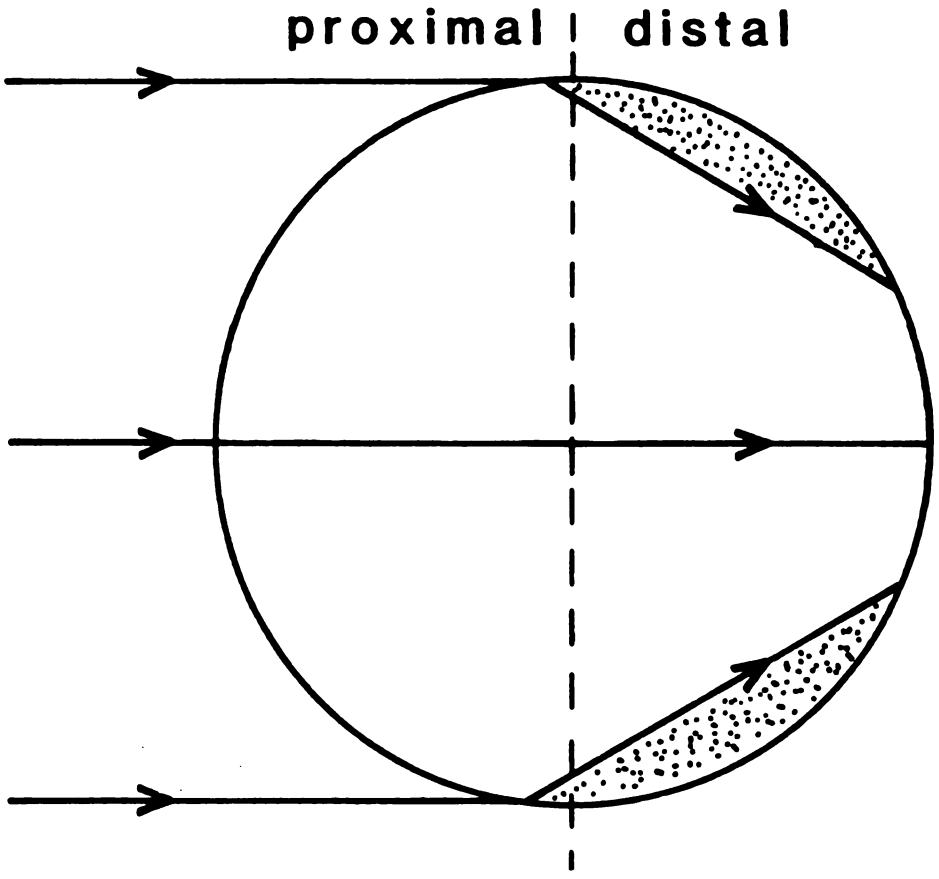

Figure 24. Schematic drawing of the cross section of a slug exposed to unilateral light. The lines represent light rays from a light source to the left of the slug. Because of refraction at the the slug-air interface, the light is focused onto the distal side of the slug.

above a certain intensity further increases in intensity do not change the magnitude of the response. This phenomenon is called saturation and is apparent in the photoresponses of the D. discoideum strains which demonstrate unidirectional phototaxis (Figure 15,23). Saturation can result when any molecule in the response pathway is unable to increase its level of response with an increase in the activity of the previous step. In photoresponses, it is often assumed that saturation occurs when an activated photoreceptor is unable to return to its ground state before the arrival of the next photon. Therefore the number of photons absorbed per unit time and per unit area reaches a maximum and does not increase even with an increase in the number of incoming photons. The following model uses the saturation phenomenon to explain bidirectional phototaxis.

In D. discoideum if a step in the response pathway on the distal side of the slug saturates, then with increasing light intensity the difference between the response pathway's output on the proximal side and the pathway's output on the distal side will decrease. For example, if saturation occurs on the pigment level, when the photoreceptors on the distal side are just saturated the receptors on the proximal side are still receptive to incoming photons. The apparent difference in intensity across the slug, usually generated by the lens property, would then diminish with still increasing light intensities. Because of the lens effect and the dark wings it produces on

the distal side (Figure 24), more of the proximal side of the slug is able to participate in a photoreaction. This difference should increase the availability of the response-limiting molecule on the proximal side thereby raising the intensity level at which saturation occurs. Again, if it is assumed that the photoreceptor is the limiting molecule, the proximal side will have more photoreceptors in the light field and will thereby saturate at a higher light intensity than the distal side. Therefore, at high light intensities, the side of the slug with the greatest apparent intensity of response would be the proximal side and the slug would tend to turn away from the light source, i.e., exhibit negative phototaxis.

The ability of the slug to focus the incoming light via the lens effect is related to the angle of the slug with respect to the light source. If the slug is crawling directly toward the light, the lens property is ineffective because it does not increase the light intensity on one side of the slug with respect to the other. The efficiency of the lens property, for phototaxis, is maximal when the slug is perpendicular to the incoming light. Therefore by changing its angle with respect to the light source, the slug can alter the magnitude of the positive and negative phototactic responses. The slug would then tend to crawl at the angle at which the tendency to turn away from the light is balanced by the tendency to turn toward the light. The result would be bidirectional phototaxis.

The above model assumes that the molecule responsible for saturation is evenly distributed within the slug and that the efficiency of the 'lens' decreases monotonically as the slug turns toward the light source from a position perpendicular to it. This last assumption is currently being checked.

This model explains the dependence of bidirectionality on light intensity, i.e., bidirectionality is only expressed at saturating light intensities. Any external factor which affects the magnitude of the phototactic response should alter the angle at which the slugs crawl with respect to the light source. This is consistent with the observation that the addition of charcoal to the agar or the addition of a slug repellant, STF, changes the angle at which the slugs migrate (15). If the slugs are impregnated with neutral red, the angle at which the slugs migrate with respect to the light source is altered (M. Schneider and D. Häder, personal communication). The dye acts as a screen and decreases the amount of light which reaches the distal side. With relatively more light being absorbed on the proximal side than on the distal, the tendency to turn away from the light should increase. If the model is correct, this should act to increase the angle at which the slugs migrate with respect to the light source. This is what is observed.

This model does not readily explain the relationship between positive thermotaxis and phototaxis, why the bidirectionality is not observable in the response of the

parental strain, or why at even higher light intensities some mutant slugs again appear to crawl directly toward the source. However, it appears as if the positive thermotactic response acts to draw the slugs toward the light source such that the tendency to turn away from the source is never expressed in the strains which demonstrate normal positive thermotaxis. The tendency of the mutants which display bidirectional phototaxis to crawl toward the light source at very high light intensities may be the result of their residual positive thermotactic response. A knowledge of the positive thermosensory and photosensory pathways on the molecular level will probably be necessary before the relationship between the two responses is understood. However, this knowledge is not necessary to further test the model summarized in this section.

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