Thermotaxis by pseudoplasmodia of *Dictyostelium discoideum*

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**ABSTRACT**  The temperature dependence of migration rate and of the thermotactic sensitivity of pseudoplasmodia of *Dictyostelium discoideum* has been measured. Migration rate increases with temperature to 20 °C, is temperature insensitive from 20 °C to 27.5 °C, and decreases with temperature to 29 °C, above which point migration ceases. However, pseudoplasmodia formed from cells grown at 23.5 °C are thermotactic only from 22 °C to 27.5 °C. Thus, a temperature dependence of migration rate is not sufficient to explain thermotaxis. Because random lateral movements by the pseudoplasmodia have not been observed, the measurement of the temperature gradient appears to be spatial rather than temporal, with a half-maximal thermotactic response to a temperature gradient of about 0.04 °C/cm, or 0.0004 °C/mm across an average pseudoplasmodium. Thermotactic sensitivity is adaptive, with pseudoplasmodia formed from cells grown at 20 °C capable of thermotaxis at temperatures lower than cells grown at 23.5 °C.

Amoebae of the cellular slime mold *Dictyostelium discoideum* aggregate to form pseudoplasmodia which contain 10^7-10^8 cells surrounded by a slime sheath, and which can migrate under suitable conditions for up to 10 cm before forming fruiting bodies (1). Migrating pseudoplasmodia are positively phototactic and positively thermotactic (2). Although phototactic migration in *D. discoideum* has recently received considerable attention (3-6), the thermotactic response of the organism has not been studied since the original observation of thermotaxis by Bonner (2). One can easily visualize a specific biological response to light that is absorbed by a specific photoreceptor pigment. In contrast, the absorption of heat is inherently non-specific. Nevertheless, an absorption of heat must precede the specific biological response of thermotaxis. The purpose of this study was to examine the mechanism whereby pseudoplasmodia of *D. discoideum* respond to thermal gradients.

**MATERIALS AND METHODS**

*D. discoideum* strain NC-4 was used in most experiments. Cells were grown in association with *Klebsiella aerogenes* for 48 hr as described by Sussman (7). The cells were then harvested and freed of bacteria by washing three times with a salt solution described by Bonner (8) and suspended in a phosphate buffer solution at 10^6 cells per ml (7). A 10 μl drop of cells was deposited in the center of a petri dish containing sterile, nonnutrient aqueous agar (2% wt/vol). After 15-16 hr incubation at 23.5 °C in darkness, aggregation had proceeded with the formation of migrating pseudoplasmodia. Alternatively, in a few experiments, *D. discoideum* strains Ax-2, and L-25 (4) were used. Strain L-25 was cultured in the same manner as strain NC-4, while strain Ax-2 was cultured axenically in liquid shake culture in a medium containing 10 g of glucose, 5 g of Difco yeast extract, and 10 g of Baltimore Biological Laboratories trypcase in 1000 ml of 2 mM potassium phosphate buffer at pH 6.5. Exponentially growing Ax-2 cells were collected by centrifugation, washed, and deposited on nonnutrient agar as above.

Temperatures were measured with a Yellow Springs Instrument Co. Tele-thermometer with a 5 mm diameter contact thermistor probe, a copper-constantan thermocouple in conjunction with a microvolt meter, or with a precision mercury-in-glass thermometer.

The incubation temperature for cultures in petri dishes was controlled by placing the dishes on a 2.5 cm × 11 cm × 36 cm aluminum block with recesses for the dishes and with holes bored lengthwise through each side of the block (Fig. 1). Water from a constant-temperature water bath was circulated through each side of the block; the latter was surrounded by a minimum of 10 cm of styrofoam and placed in a dark room. The temperature of each side of the petri dish could be varied from 12 °C to 35 °C and was constant to ±0.05 °C for 24 hr. Temperature gradients were established across petri dishes by setting the two sides of the aluminum block to the different desired temperatures. Temperatures measured across a petri dish on an imposed 1.1 °C/cm gradient varied linearly with distance across the dish.

**RESULTS**

Transverse sensitivity to heat

A change in direction of migration requires unequal rates of migration by the two sides of a pseudoplasmodium. Phototaxis in *D. discoideum* is known to involve a lens effect; that is, unilateral light is focused onto the distal cells of the pseudoplasmodium because of the refraction at the proximal pseudoplasmodium-air interface (3, 5). The highest relative light intensity is thus in the distal half of the pseudoplasmodium, and results in an acceleration in the migration rate of this distal pseudoplasmodium half and thus a turn of the pseudoplasmodium toward the light source. This interpretation is based on the finding that illumination of a single side of a pseudoplasmodium by a vertical light beam results in migration away from the illuminated side (3, 5), and is strengthened by the demonstration that pseudoplasmodia migrate faster in light than in darkness (5).

Thermotactic pseudoplasmodia turn toward the warmer side in a heat gradient. Heat cannot be focused by a pseudoplasmodium onto the distal or cooler side of the organism. (Heat *per se* cannot be focused; the heat transferred as infrared radiation from an environment at 20 °C-30 °C would have a maximum wavelength at about 9.5-10 μm, and would be absorbed mainly on the proximal or warmer side of the pseudoplasmodium.) It must then be assumed that the heat gradient is detected on the proximal or warmer side of the pseudoplasmodium, and that the elevated temperature causes a reduction in the migration rate at this side relative to the cooler side of the pseudoplasmodium. Thus, while pseudoplasmodia should turn away from a unilaterally absorbed vertical microbeam of visible light, they should turn toward such a microbeam of heat-producing infrared radiation. We confirmed this prediction by irradiating pseudoplasmodia with a microbeam of visible or infrared radiation on one side of the pseudoplasmodium. A mi-
Microbeam of visible light is partially absorbed by the photoreceptor of phototaxis (5, 9) and the pseudoplasmodia migrate away from the microbeam (Table 1). An infrared microbeam is absorbed minimally by the photoreceptor pigment of phototaxis (8) but is absorbed by water in the medium or within the pseudoplasmodium and dissipated as heat, resulting in a localized heating of the irradiated side of the pseudoplasmodium. As predicted, the pseudoplasmodia turned toward the side of the pseudoplasmodium irradiated with the infrared microbeam (Table 1). The localized heating resulting from the microbeam irradiation of the pseudoplasmodium would be insufficient to result in any damage to the organism (based on irradiation of a cylindrical section of pseudoplasmodium 35 μm in diameter by 20 μm in depth with a beam of 2 mW·cm⁻² over an area of 3.8 × 10⁻³ cm², assuming that much less than 10% of the incident radiation >825 nm would be absorbed in a 20 μm path length).

Sensitivity of pseudoplasmodium migration to temperature

We looked for a change in migration rate with temperature that could be used to explain turns in response to heat. Pseudoplasmodia were permitted to form at 23.5° on petri dishes containing nonnutrient agar and the dishes were then placed on a constant-temperature block. For each test temperature from 14° to 29°, the pseudoplasmodia were permitted to migrate on the constant-temperature block at the test temperature for 24 hr while similar pseudoplasmodia migrated for the same time at 23.5° (not on the constant-temperature block) in darkness as a control. Following incubation, the dishes were shadowgraphed and the lengths of slime tracks left by the 10 most rapidly migrating pseudoplasmodia were measured directly from the shadowgraphs. The distance migrated, expressed as a percentage of migration by the control pseudoplasmodia, shows an increase in migration rate with increasing temperature to 20°, a range, 20°–27.5°, over which no temperature dependence could be observed; a narrow range, 27.5°–29°, over which migration decreased with increasing temperature; and no migration above 29° (Fig. 2). Migration rate on the control plates was greater than that on the constant temperature block when the incubation temperature for the two conditions was the same. This reduction of migration on the constant temperature block may have resulted from a change of aeration or humidity within the styrofoam incubation chamber.

A temperature-dependent decrease in migration rate could account for a turn of the pseudoplasmodium toward the region of higher temperature. Although the only temperature region over which migration rate decreased with increasing temperature was 27.5°–29°, later results (Fig. 5) showed that thermotaxis occurred over the temperature range 22°–28°, where no temperature dependence of migration rate was observed. However, the error bars in Fig. 2 are large enough to conceal a small decrease in migration rate with increasing temperature from 22° to 28°. We therefore measured the temperature-dependent change in migration rate that would be required to account for the change in migration rate of the two sides of a pseudoplasmodium turning in response to a temperature gradient. Pseudoplasmodia were directed for 12 hr along a 0.44°/cm gradient. The petri dish was then rotated 90° and the pseudoplasmodia were permitted to migrate for an additional 12 hr. For each of 25 pseudoplasmodium tracks, the radius of the turn and the width of the pseudoplasmodium were measured. The percentage change in path length for the inside and outside edge of the pseudoplasmodia was calculated from these...
data as 6.4% (standard deviation = 3.4%). Thus, the anticipated difference in migration rates of the two sides is 3–10% with a thermal gradient that gives maximal thermotaxis (0.44°/cm). Assuming a 0.1 mm pseudoplasmodium width, the 3–10% difference in migration rate across the pseudoplasmodium results from a temperature difference across the pseudoplasmodium of 0.0044°. A 300–1000% change in migration rate of the pseudoplasmodium would then be expected for a change in temperature of 0.44°. Such a temperature-dependent change in migration rate was not observed (Fig. 2), and a temperature-dependent decrease in migration rate is therefore insufficient to explain thermotaxis.

**Thermotaxis with different thermal gradients**

The dependence of thermotaxis on the thermal gradient was determined quantitatively by measuring thermotaxis on different thermal gradients with the midpoint of the gradient and of the petri dish kept constant at 23.5°. Pseudoplasmodia incubated isothermally migrate randomly from the inoculum drop (Fig. 3A), while those incubated on a thermal gradient migrate toward the warmer side of the petri dish or “up” the thermal gradient (Fig. 3 B and C). A thermotaxis index was calculated as 360°/θ, in which θ is the angle subtended by the migration area. Thus, in Fig. 3A, migration is random, θ is 360°, and the thermotaxis index is 0°. Similarly, in Fig. 3B, migration is somewhat directed, θ is 105°, and the thermotaxis index is 235°, while in Fig. 3C migration is strongly directed, θ is 40°, and the thermotaxis index is 320°. The sensitivity to a given thermal gradient varied somewhat from experiment to experiment, so the test results for any given thermal gradient were corrected for constant thermotactic response to a control 0.22°/cm thermal gradient (23°–25°), and then calculated as a percentage of the maximum thermotactic response obtained. Relative thermotaxis increases with increasing thermal gradient from 0°/cm to 1.1°/cm with a half-maximal response at about 0.04°/cm (Fig. 4) or 0.0004° across a 0.1 mm pseudoplasmodium.

**Dependence of thermotaxis on temperature**

It is important to examine the temperature range over which the high sensitivity of thermotaxis (50% response to a 0.04°/cm gradient) is achieved because the temperature sensitivity for thermotaxis may also be a measure of the temperature sensitivity for the thermotransducer mechanism. Petri dishes containing pseudoplasmodia on nonnutrient agar were incubated as above in darkness with a temperature gradient of 0.11°/cm, but with the midpoint of the gradient and of the petri dish at differing temperatures. Control dishes were incubated on a 0.11°/cm gradient (24°–25°) and isothermally at 23.5°. Shadowgraphs following a 24 hr incubation were measured as before to calculate a thermotaxis index, which was then expressed as a percentage of the thermotaxis index of the culture incubated on the isothermal control dish.

The results (Fig. 5, solid line) show the relative capability of pseudoplasmodia to respond to a 0.11°/cm gradient as a function of the midpoint temperature of the gradient. The pseudoplasmodia are thermotactically sensitive over the temperature range from 22° to 28° but not above or below that range. The total range for thermotaxis to a 0.11°/cm gradient is less than 6°. Pseudoplasmodia formed from cells grown at 20° and aggregated at 23.5° for 16 hr (cells aggregated significantly slower at 20° than at 23.5°) were thermotactic at lower temperatures than were pseudoplasmodia formed from cells grown and aggregated at 23.5° (Fig. 5, broken line). Similar temperature-adaptation results were obtained with strain NC4 grown on bacteria and strain Ax-2 grown axenically, indicating that the thermoadaptation is not a function of the bacteria but rather is a function only of the slime mold. The nonphototactic mutant L-25 exhibits unimpaired thermotaxis, demonstrating again that the sensory inputs for phototaxis and thermotaxis are separate.

**DISCUSSION**

The temperature sensitivity of pseudoplasmodia indicated by the data in Fig. 4 confirms the sensitivity reported by Bonner (2) and warrants special attention. The pseudoplasmodium must

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**FIG. 3.** Redrawn shadowgraphs of Dictyostelium cultures that were exposed during incubation to different thermal gradients. i.d. = inoculum drop; p = pseudoplasmodium; s.t. = slime trail. (A) No thermal gradient, culture temperature 23.5°; (B) 0.056°/cm thermal gradient from 23.25° to 23.75°; (C) 0.44°/cm thermal gradient from 21.5° to 25.5°. Horizontal arrows above the drawings point toward the warmer side of the gradient.

**FIG. 4.** Relative thermotactic response of pseudoplasmodia exposed to different thermal gradients with the midpoint of the gradient and of the petri dish at a constant (23.5°) temperature.

**FIG. 5.** Relative ability of pseudoplasmodia to respond to a 0.11°/cm thermal gradient as a function of the temperature at the midpoint of that gradient. O—O, cells grown and aggregated at 23.5°; △—△, cells grown at 20° and permitted to develop for 16 hr at 23.5°.
be measuring temperature either with respect to distance or with respect to time of migration in moving toward the warmer portion of its environment. The sensitivity of bacteria to chemical gradients has been partially explained by the evidence that bacteria measure such gradients with respect to time rather than with respect to distance (10). The bacterium accomplishes this by a periodic tumbling, the frequency of which is inversely related to the change in chemical concentration, and the random choice of a new path direction following a given tumble. Thus, the direction of path of the bacterium is randomly elected but the distance traveled along that path before further tumbling is greater when the direction is toward an optimum concentration than when the direction is away from that concentration (10). We have been unable to find any evidence to support a temporal measurement of temperature by pseudoplasmodia of *Dictyostelium*. The pseudoplasmodia exhibit little if any random directional movement in a thermal gradient. Further, the general path of the occasional pseudoplasmodium that migrates “down gradient” before turning to migrate “up gradient” is indistinguishable in the two directions. That is, neither the migration rate nor the frequency of lateral movements is observably changed along the two paths. When pseudoplasmodia were permitted to migrate first in one direction along a 0.44°/cm gradient and then along the same gradient rotated 90° to the first, 90% of the pseudoplasmodia responded with their first observable turn “up gradient.” We cannot rule out a very small (<5 μm) random lateral movement with time, and so cannot eliminate the possibility of a temporal heat measurement, but our experimental results can be more easily explained by a temperature measurement that is spatial than by one that is temporal.

Other microorganisms also possess temperature-sensing systems. The slime mold *Physarum polycephalum*, the nematode *Caenorhabditis elegans*, and the protozoan *Paramaecium caudatum* all exhibit some specific thermosensitive migration behavior (11-13), but the migration in these organisms is toward a temperature optimum, in contrast to *Dictyostelium*, for which there is no optimum temperature at which the organism will accumulate. Thermoadaptation has been observed in *Caenorhabditis* (12) and *Paramaecium* (14), although the sensitivities to temperature in these organisms are much lower than in *Dictyostelium*. The thermosensory transducer is not known in any of these systems but could ultimately prove to be the same. Thermotaxis in *Dictyostelium* provides a promising system for the study of thermosensory transduction in eukaryotic organisms, particularly because of the ability of the pseudoplasmodium to detect extremely small temperature differences. Such a sensitivity permits one to eliminate some potential mechanisms for measuring temperature. For example, an organism might sense relatively large temperature differences through the normal temperature-dependent changes in an enzymatically controlled metabolic reaction. However, it would be difficult for such a mechanism to provide for the measurement of temperature differences of 0.0004°. Moreover, the low thermotactic sensitivity over the temperature range from 19.5° to 21.5° (Fig. 5), in which migration rate is relatively unimpaired (Fig. 2), is evidence that thermotaxis in *Dictyostelium* does not operate via a general thermal effect on the metabolism involved in migration.

One may assume that many steps are involved in thermotaxis, from heat detection to the physiological response of turning. The heat gradient detection mechanism or thermotransducer can be considered to involve a substance that undergoes a temperature-dependent change of state. The temperature sensitivity curve for thermotaxis (Fig. 5) is a measure of the physiological capability of a pseudoplasmodium to sense a specific temperature gradient at different temperatures. In the absence of other limiting steps, this physiological capability to sense a temperature gradient is a representation of the ability of the thermotransducer to change its state over that temperature range. Thus, the temperature sensitivity curve for thermotaxis provides a potential identifying characteristic of the thermotransducer itself. This argument may not be valid in the presence of secondary limiting steps. For example, any of the steps between the initial heat detection and the physiological turning response could contribute to the temperature sensitivity curve for thermotaxis if that step: (a) exhibited a strong temperature dependence over the temperature range for thermotaxis and (b) imposed a kinetic limitation on the entire transducer-to-response process. It would be difficult to establish that there are no such secondary limiting steps. However, migration rate is temperature insensitive over the temperature range of thermotaxis, and thus, at least the metabolic steps in migration rate are not secondary limiting steps for thermotaxis.

Any transducer mechanism for thermotaxis must meet the following criteria: the mechanism must provide for a relatively high gain or high sensitivity to temperature change; it must be capable of measuring temperature differences only over a fairly narrow temperature range; and it must provide for thermal adaptation to growth temperature. The narrow thermal response range is consistent with the possibility that the thermotransducer is a specific substance or structure (e.g., a protein or a lipid) that undergoes a phase transition over a relatively narrow temperature range. The phenomenon of thermal adaptation supports the hypothesis that the substance is a lipid in a membrane matrix, because only the lipid components in membranes have been shown to exhibit such temperature adaptation (15-17).

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