

Thermosensing of Thermotactic Mutants, *Dictyostelium discoideum* Amoebae in Vegetative Stage

Hong, Choo Bong*

(MSU/Doe Plant Research Lab., Michigan State University, East Lansing, Michigan, U.S.A.)

ABSTRACT

Temperature responses of amoebae of thermotactic mutants have been investigated. Amoebae of the mutant strain HO 428 showed positive thermotaxis which is strong at lower temperatures and drops sharply above the growth temperature of amoebae. The temperature response of HO 428 amoebae was not affected by the length of amoebae on the gradients. HO 596 amoebae seemed to have both positive and negative thermotactic responses shortly after food depletion. Longer exposure of these amoebae on the thermal gradients induced a stronger negative response at lower temperatures and an apparent positive response at higher temperatures. A similar change could be observed in HO 1445 amoebae. Based on the steady positive thermotactic response by HO 428 amoebae and the mode of change in temperature response at higher temperatures, 24°C and 26°C, by HO 596 amoebae, a model for the temperature response of vegetative *Dictyostelium discoideum* amoebae, strain HL 50, has been proposed. The main features of the model are: a positive response at the thermal gradients with midpoint temperatures lower than the growth temperatures of amoebae and a negative response above it.

INTRODUCTION

Amoebae of *Dictyostelium discoideum* feed on bacteria in the mulch of the forest floor. Once the food is depleted, they follow a developmental cycle which includes formation of a pseudoplasmodium and a fruiting body (Raper, 1940). During this life cycle, *D. discoideum* has two motile stages, the amoebae and the slug, which are able to respond to environmental stimuli by showing oriented movements.

Amoebae of *D. discoideum* are positively chemotactic to folic acid (Pan *et al.*, 1975) and cAMP (Robertson *et al.*, 1972), negatively chemotactic to an unidentified "repellent (s)" (Bonner, 1977), and positively or negatively phototactic (Hong *et al.*, 1981). Pseudoplasmodia of *D. discoideum* are negatively chemotactic to "STF" (Fisher *et al.*, 1981), phototactic (Poff *et al.*, 1973), and positively and negatively thermotactic (Whitaker and Poff, 1980).

*Present address; Dept. of Chemistry, Texas Tech. University, Lubbock, Texas, U.S.A.

In the previous report (Hong *et al.*, 1983), thermotaxis of *D. discoideum* amoebae was presented. Amoebae of strain HL 50, grown at 23.5°C, showed only positive thermotaxis throughout the thermal gradients checked shortly after food depletion. The temperature response curve of amoebae at this time point (3 hr) showed a strong positive thermotactic response between 18° and 23°C, while above 23°C, there was a drop in the strength of positive thermotaxis. Random responses could be observed on the thermal gradients with low midpoint temperatures at 6 hr and 9 hr time points, and became negative beyond 9 hr. Negative thermotaxis on the thermal gradients with low midpoint temperature became apparent after 12 hr on the gradients, and grew stronger as exposure time on the gradients increased. Based on these data, it has been proposed that the vegetative amoebae of *D. discoideum* are capable of a positive thermotaxis, and the negative thermotactic response at low temperature is probably induced by food depletion and/or exposure of the amoebae on the thermal gradients.

In this work, thermotactic responses of mutants derived from HL 50 were checked at three different time intervals on the thermal gradients. The results were then used to postulate the way vegetative amoebae respond on the thermal gradients.

Thermotaxis mutants used in those works were originally selected based on the different slug thermotactic responses. HO 428, HO 596 and HO 813 have been isolated by Schneider *et al.* (1982), while HO 1445 was isolated as mentioned in Hong (1983).

MATERIALS AND METHODS

The characterization of the thermotaxis of *D. discoideum*, strains HO 428, HO 596, HO 813 and HO 1445, was conducted as follows: amoebae were grown in association with *K. aerogenes* (Sussman, 1966) for about 20~24 hr at $23.5 \pm 0.3^\circ\text{C}$ in the dark. At this time, the bacteria had been cleared from about 80% of the agar plate. The amoebae were freed of bacteria by washing with buffer, aliquotes of the washed amoebae were mixed in suspension with washed charcoal, and the mixture of amoebae and charcoal was linearly spotted on the agar covering a glass microscope slide by means of the micromanipulator. The slides were then placed on a temperature gradient, $0.22^\circ\text{C}/\text{cm}$, and left there for 3, 6, and 16 hr. After the exposure on the thermal gradient, the response was measured, $\Delta\%$ was calculated, and the thermal response curves were plotted. Vertical error bars represent 95% confidence intervals. See Hong *et al.* (1982) for detailed methods.

RESULTS

Amoebae of *D. discoideum*, strain HO 428, grown at 23.5°C showed positive thermotaxis throughout the entire temperature range after 3 hr on the gradients. Stronger positive responses were observed at the lower temperatures (Fig. 1A). These positive respon-

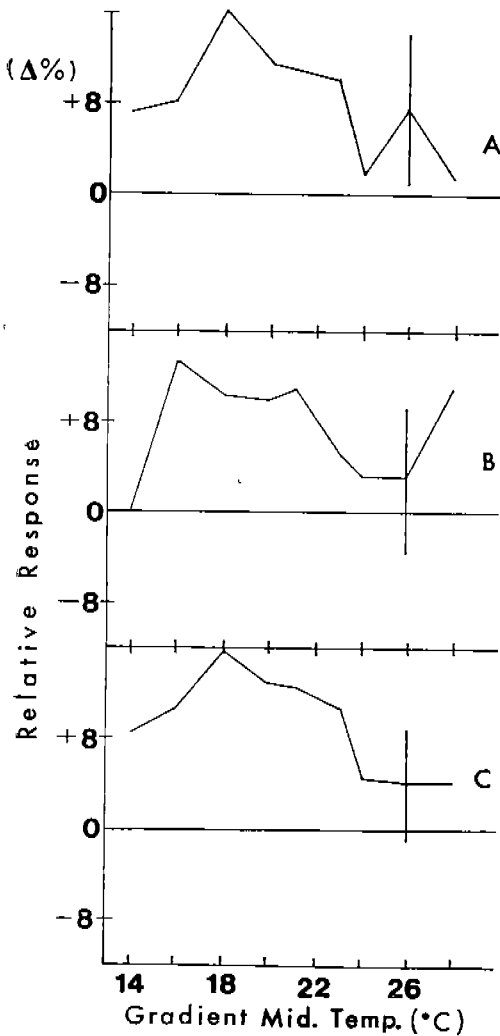


Fig. 1. Temperature response curves for strain HO 428 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.

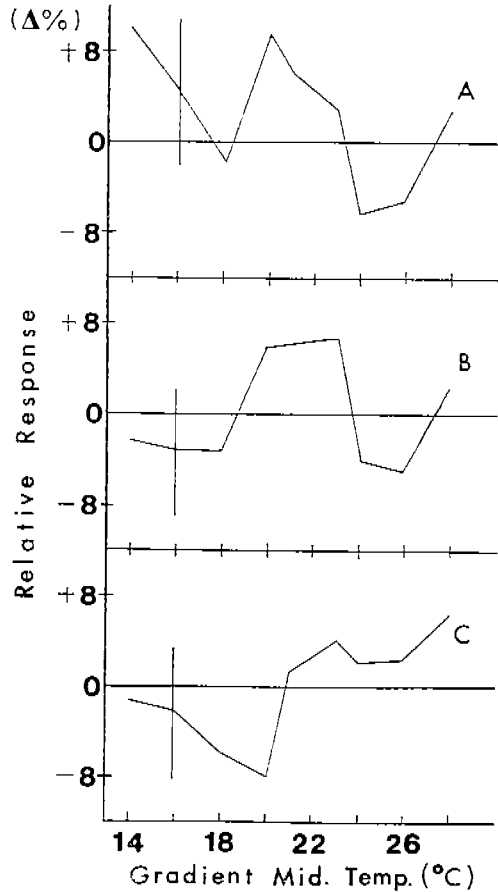


Fig. 2. Temperature response curves for strain HO 596 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.

ses continued at 6 hr (Fig. 1B) and 16 hr (Fig. 1C) with no significant differences.

Amoebae of strain HO 596 showed complicated thermotactic responses after 3 hr on the gradients. Positive thermotactic responses were observable on the thermal gradients with the midpoint temperatures lower than 23°C except at 18°C, and negative thermotactic responses were also seen at 24° and 26°C (Fig. 2A). Positive thermotactic res-

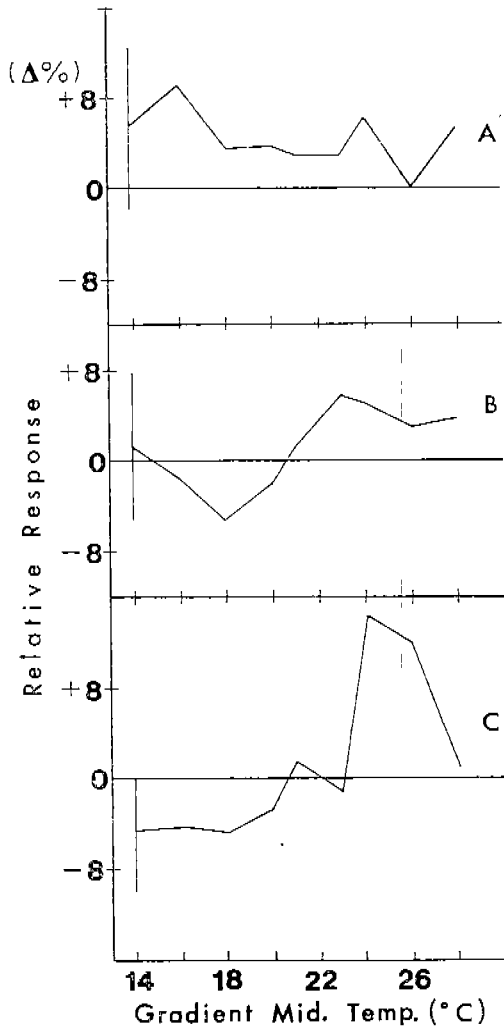


Fig. 3. Temperature response curves for strain HO 813 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.

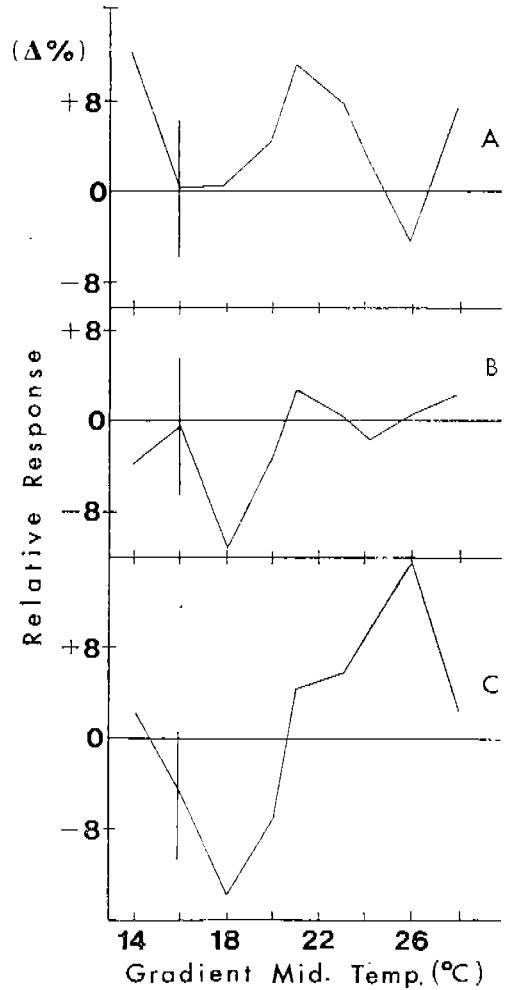


Fig. 4. Temperature response curves for strain HO 1445 amoebae grown at 23.5°C and exposed for different lengths of time on the thermal gradient. A) after 3 hr, B) after 6 hr, and C) after 16 hr. Vertical bars represent the 95% confidence interval, and each datum point represents the average of 8 replicates.

ponses at low temperatures, 14° and 16°C, were quickly diminished after 6 hr, but little change was observable from other midpoint temperatures (Fig. 2B). This change of response (a shift in the thermal response curve toward the negative direction at lower temperatures) was more apparent at the 16 hr time point. After 16 hr, the temperature response curve showed negative thermotactic responses at lower temperatures and weak

positive thermotaxis above 21°C with a transition point from negative to positive thermotaxis at about 20.8°C (Fig. 2C). Noticeable changes which occurred during this time course were more apparent negative thermotactic responses at low temperatures and absence of the previously existing negative thermotactic responses at 24° and 26°C.

D. discoideum amoebae, strain HO 813, showed no negative thermotactic responses over the entire temperature range after 3 hr on the gradients. Although the strength of the positive response is much weaker than the parental strain HL 50 and mutant HO 428, HO 813 amoebae exhibited positive thermotactic responses on most temperature gradients (Fig. 3A). After 6 hr, negative thermotaxis is apparent at 18°C, but not much change was observed in the strength of positive thermotactic response on the gradients with higher midpoint temperatures, and the transition point from negative to positive thermotaxis was at about 20.8°C (Fig. 3B). During the time interval between 6 and 16 hr, negative thermotaxis was observed at a broader temperature range, positive thermotaxis was strengthened at temperature gradients with higher midpoint temperatures and there appeared to be a small shift in the transition point. After 16 hr, negative thermotactic responses were seen at the gradients below 21°C while positive thermotaxis was observable at the gradients above 23°C (Fig. 3C).

After 3 hr on the gradients, HO 1445 amoebae showed complicated temperature responses similar to those of HO 596 amoebae. Positive thermotaxis at 14°, 20°, 21°, 23° and 28°C and a weak negative thermotactic response were observed at 26°C (Fig. 4A). During the next 3 hr, the temperature response curve was shifted downward on most gradients with low midpoint temperatures. Negative thermotactic responses were observable below 21°C after 6 hr (Fig. 4B). Between 6 hr and 16 hr time interval, strong positive thermotactic responses were given by amoebae at the higher midpoint temperatures. The maximum negative response was seen at 18°C, and the maximum positive response was at 26°C. The transition point from negative to positive thermotactic responses was at about 20.6°C (Fig. 4C).

DISCUSSION

The low density of amoebae (less than 3×10^3 cells/cm²) used in these experiments should be low enough to eliminate any possible chemotactic effects (Gingle, 1976; Grutsch and Robertson, 1978) in amoebal movement of these mutants.

Early in development, *D. discoideum* amoebae, mutant strain HO 428, show strong positive thermotactic responses at temperatures lower than the growth temperature of the amoebae, while above the growth temperature, the thermotactic responses are very weakened. Extending the exposure on the temperature gradients to 16 hr did not induce noticeable differences from the temperature response curve obtained after 3 hr. Thus, in the mutant strain, HO 428, development does not affect thermotactic response in amoe-

bae. Based on this, one can postulate a temperature response curve of the growing amoebae of the mutant strain, HO 428, which is quite similar to the ones appearing in Fig. 1.

In another mutant strain, HO 596, recently starved amoebae seemed to have both thermotactic responses, i.e., positive and negative. After 3 hr on the gradients, HO 596 amoebae were positively and negatively thermotactic. As the exposure time on the gradients increased, negative thermotaxis was induced at lower temperatures and diminished at higher temperatures. As development proceeds negative thermotactic responses at higher temperatures, 24°C and 26°C, diminished; this change probably supports the existence of negative thermotaxis in the vegetative amoebae at higher temperatures. This postulated negative thermotaxis of the vegetative amoebae at higher temperatures probably is at least as strong as the responses seen from the temperature response curves at 3 and 6 hr time points. This suggests that growing amoebae, strain HO 596, would have a negative thermotactic transduction path as well as a positive path. Mutant HO 1445 amoebae showed a temperature response and change of the response along development similar to that of HO 596. Negative thermotaxis increases in strength on the gradients with lower midpoint temperatures, and positive thermotaxis becomes more apparent at higher temperatures as development proceeds. Thus, the indication from these two mutants, HO 596 and HO 1445, is that vegetative amoebae of *D. discoideum* have the capacity for both positive and negative thermotaxis. The temperature ranges of the positive and negative thermotaxis expected from the results of these two mutants would be below the growth temperature and above the growth temperature of the amoebae, respectively, and this feature may suggest a mechanism for seeking an optimal environment in growing *D. discoideum* amoebae, i.e., vegetative amoebae gather at their growth temperature.

The proposed temperature response curve of the growing amoebae of the parental strain, HL 50 is similar to the temperature response curve of HO 428 amoebae. Both have strong positive thermotactic responses below the growth temperature of the amoebae and a weaker response above that temperature. The results with HO 596 and HO 1445 strongly suggest negative thermotaxis in the vegetative amoebae of *D. discoideum*. Based on the temperature response curves from these mutants one can suggest the following points:

- 1) HO 428 is a mutant with a defect in negative thermotactic transduction path in amoebae as it shows only positive thermotaxis.
- 2) HO 596 and HO 1445 are mutants which have mutations in the development of positive thermotaxis at higher temperatures, 24° and 26°C, such that they have delayed expression of positive thermotaxis at these temperatures, i.e., the observed negative thermotaxis at higher temperatures in these mutants is due to a delayed development of the positive thermotactic response at these temperatures. This suggests that growing HL 50 amoebae also have a capacity of negative thermotaxis at higher temperatures,

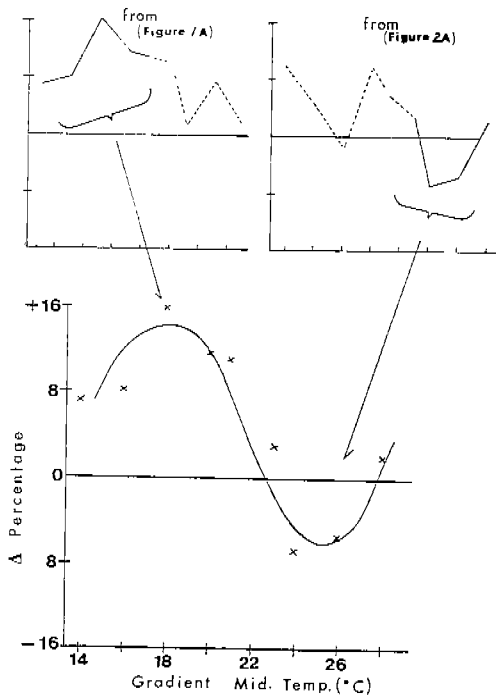


Fig. 5. Proposed temperature response curve of *D. discoideum* amoebae at the vegetative growth stage. This model is primarily based on the positive thermotaxis of amoebae, strain HO 428, at lower temperatures (Fig. 1A) and negative thermotaxis of amoebae, strain HO 596, at higher temperatures (Fig. 2A)

but the first three hours on the thermal gradient is long enough to induce a positive thermotaxis at these temperatures.

3) Positive thermotactic responses at 16° and 18°C in growing HO 596 and HO 1445 amoebae are rapidly diminished compared to other strains after food depletion.

Based on these results and assumptions, a tentative temperature response curve of vegetative HL 50 amoebae may be proposed. Its main features would be a positive thermotactic response at the temperature gradients lower than the growth temperature of amoebae with the maximum at about 18°C and a negative thermotactic response above the growth temperature (Fig. 5). Probably the strength of negative thermotaxis would be weaker than that of positive thermotaxis because of its narrower and weaker responses as can be seen in Fig. 2A and 4A.

In *Caenorhabditis elegans*, a nematode which shows thermotaxis (Hodgcock and Russell, 1975), and the slug of *D. discoideum* (Whitaker and Poff, 1980), two sensors, one for positive thermotaxis and

the other for negative thermotaxis, have been proposed to accommodate their thermotactic responses (actually these two organisms show an opposite way of responding on the thermal gradients; thermotaxis of *C. elegans* can be called an optimum seeking process, and thermotaxis of *D. discoideum* slug has been called as an "optimum leaving" mechanism) (Schneider *et al.*, 1982). In these two-thermosensor models, the apparent response will be determined by the input from the two sensors. Based on the model proposed here for vegetative amoebae of *D. discoideum*, growing amoebae have the ability to respond positively or negatively on the thermal gradients dependent on the temperature. The shift of sign of response from positive to negative would occur at about the growth temperature of the amoebae. This sign reversal could be a result of two competing sensors as suggested in *C. elegans* and *D. discoideum* slug, or could be a result of a difference in stimulus strength which suggests only one sensor, examples of which can be found in photoresponses (Forward, 1976; Häder and Poff, 1979). More

evidence is necessary to decide which is the case for vegetative amoebal thermosensing.

Amoebae of mutant strain HO 813 showed only weak positive thermotactic responses at most temperatures after 3 hr. After 6 hr, HO 813 amoebae showed a negative thermotactic response at around 18°C, but there was no significant change at higher temperatures. Weak responses at the 3 hr time point at lower temperatures could be a result of a transition from stronger positive thermotaxis at 0 hr time point i.e., growing amoebae, to negative thermotaxis at 6 hr time point, or, it is also possible that recently starved HO 813 amoebae have a mutation at a signal amplification path which results in only weak responses.

In contrast with the sensing of other environmental parameters, thermosensing usually demands consideration of the whole organism in signal reception, because all of the molecules can gain or lose heat. This nonspecificity in receiving signals makes it a great deal more difficult to identify the thermoreceptor. Amoebae of HO 428 show no significant change in their thermoresponses as they develop. This stable thermotactic expression of HO 428 may be an excellent control system when dealing with thermoreceptors. The increase of negative thermotaxis in other strains can be compared with the steady positive temperature response of HO 428 at the level of the whole organism or at a molecular level.

Acknowledgements. The author would like to express a sincere appreciation to Dr. Ken Poff for his helpful discussion. The technical assistance of Douglas DeCaetano, Elizabeth Wietor and Therese Best is greatly acknowledged.

This work was supported by United States Department of Energy contract DE-AC02-76ERO-1338.

摘 要

아메바의 走溫性 突然變異體들에 대한 溫度反應을 영양생장 단계에서 조사하였다. 突然變異株 HO 428은 생장온도보다 낮은 온도에서 강한 양성 주온성을, 그리고 생장온도 보다 높은 온도에서는 급격히 감소되는 주온성을 나타내었다. HO 428 아메바의 온도 반응은 溫度勾配에서 처리한 시간의 길이에 의하여 영향을 받지 않았으나 HO 596 아메바의 경우, 양분용 고갈시킨 직후에는 양성 및 음성외의 두가지 주온성 반응을 보이는 것 같았다.

溫度勾配에서 오랫동안 처리된 이 아메바들에서는 낮은 온도에서 강한 음성반응이 유도되었고 높은 온도에서 뚜렷한 양성반응이 유도되었다.

HO 1445 아메바에서도 유사한 변화가 관찰되었다. HO 428 아메바에 의한 일정한 양성 주온성 반응과 HO 596 아메바에 의한 24°C와 26°C에서의 높은 온도 반응의 변화방식을 근거로 하여, 營養期에 있는 아메바 *D. discoideum*, 突然變異體 HL 50의 온도반응에 대한 모델을 제시하였다. 이 모델의 주 내용은 아메바의 생장온도보다 낮은 중간 온도의 온도구배에서 양성 반응을, 그리고 생장온도 이상의 온도에서 음성 반응을 나타낸다는 것이다.

REFERENCES

- Bonner, J. T. 1977. Some aspects of chemotaxis using the cellular slime molds as an example. *Mycologia* **69**: 443~459.
- Fisher, P. R., E. Smith and K. L. Williams. 1981. An extracellular chemical signal controlling phototactic behavior by *Dictyostelium discoideum* slugs. *Cell* **23**: 799~807.
- Forward, R. B., Jr. 1976. Light and diurnal vertical migration: Photobehavior and photophysiology of plankton. *Photochem. Photobiol.* **1**: 157~209.
- Häder, D. P. and K. L. Poff. 1979. Photodispersal from light traps by amoebae of *Dictyostelium discoideum*. *Exp. Mycol.* **3**: 121~131.
- Hedgecock, E. M. and R. L. Russell. 1975. Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* **72**: 4061~4065.
- Hong, C. B., M. A. Häder, D. P. Häder and K. L. Poff. 1981. Phototaxis in *Dictyostelium discoideum* amoebae. *Photochem. Photobiol.* **33**: 373~377.
- _____, D. R. Fontana and K. L. Poff. 1983. Thermotaxis of *Dictyostelium discoideum* amoebae and its possible role is slug thermotaxis. *Proc. Natl. Acad. Sci. U.S.A.* **80**: 5646~5649.
- _____. 1983. Thermosensing and photosensing in *Dictyostelium discoideum* amoebae. Ph. D. dissertation, submitted to Michigan State University, E. Lansing, MI.
- Pan, P., E. M. Hall and J. T. Bonner. 1975. Determination of the active portion of the folic acid molecule in cellular slime mold chemotaxis. *J. Bacteriol.* **122**: 185~191.
- Poff, K. L., W. L. Butler and W. F. Loomis, Jr. 1973. Light-induced absorbance changes associated with phototaxis in *Dictyostelium*. *Proc. Natl. Acad. Sci. U.S.A.* **70**: 813~816.
- Raper, K. B. 1940. Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J. Elisha Mitchell Sci. Soc.* **56**: 241~282.
- Robertson, A., D. J. Drage and M. H. Cohen. 1972. Control of aggregation in *Dictyostelium discoideum* by an internal periodic pulse of cyclic adenosine monophosphate. *Science* **175**: 333~335.
- Schneider, M. J., D. R. Fontana and K. L. Poff. 1982. Mutants of thermotaxis in *Dictyostelium discoideum*. *Exp. Cell Res.* **140**: 411~416.
- Sussman, M. 1966. Biochemical and genetic methods in the study of cellular slime mold development. In *Methods in cell physiology*, D. Prescott(ed.), Vol. 2, pp.397~410. Academic Press, New York.
- Whitaker, B. D. and K. L. Poff. 1980. Thermal adaptation of thermosensing and negative thermotaxis in *Dictyostelium*. *Exp. Cell Res.* **128**: 87~93.

(Received August 29, 1983)