ELSEVIER

Biophysical Chemistry 92 (2001) 47–52

Biophysical Chemistry

www.elsevier.com/locate/bpc

Path finding by tube morphogenesis in an amoeboid organism

Toshiyuki Nakagaki[a,b,*], Hiroyasu Yamada[a,b,c], Ágota Tóth[d]

[a] *Local Spatio-Temporal Functions Lab., RIKEN Institute, Hirosawa 2-1, Wako, Saitama 351-0198, Japan*
[b] *Research Institute for Electronic Science, Hokkaido University, Sapporo 060-0812, Japan*
[c] *Bio-Mimetic Control Research Center, RIKEN Institute, Shimoshidami, Moriyama, Nagoya 463-0003, Japan*
[d] *Department of Physical Chemistry, University of Szeged, P.O. Box 105, Szeged, H-6701, Hungary*

Received 13 February 2001; received in revised form 29 May 2001; accepted 31 May 2001

Abstract

We have studied how the plasmodium of *Physarum polycephalum*, a large amoeboid cell, is able to track the shortest path between two selected points in a labyrinth. When nutrients are supplied at these points to a sheet-like plasmodium extended fully in a maze, the organism forms a single tube which connects the two sites via the shortest route. During the path finding, plasmodial parts in dead ends of the maze shrink and finally the tube with the minimum-length is selected from the existing possibilities. A simple cellular mechanism based on interacting cellular rhythms may describe the experimental observations. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Morphogenesis; Oscillation; Amoeboid movement; Optimization; *Physarum*; Maze

1. Introduction

Solving a labyrinth is a challenging task even for higher organisms, therefore, it may be of great interest to learn more about this capability. Babloyantz and Sepulchre [1,2] were the first to show computationally that a simple network of non-linear oscillators is also able to navigate in a maze. Showalter et al. [3] and Agladze et al. [4] have studied the path finding in actual chemical systems including the Belousov-Zhabotinsky (BZ) reaction.

A true slime mould, the plasmodium of *Physarum polycephalum*, is an amoeboid organism with sheet-like shape. This organism may be regarded as a two-dimensionally distributed biochemical reactant. Cellular activities of the plasmodium can be modeled by oscillatory reaction–diffusion type equations [5–9]. This implies that the plasmodium may also be able to solve a labyrinth [10].

Biochemical oscillators [11–13] in the plasmod-

* Corresponding author. Tel.: +81-11-706-2862; fax: +81-11-706-4967.

E-mail address: nakagaki@es.hokudai.ac.jp (T. Nakagaki).

PII: S0301-4622(01)00179-X

ium may give rise to propagating waves by spatial interactions of diffusion and advection via protoplasmic streaming. These intracellular waves can be initiated by some external stimulation including the addition of nutrients, the increase of light intensity, humidity, or temperature. The traveling wave leads to the development of a tubular structure in the sheet-like parts [14]. Therefore, the geometry of the tube network drastically changes, depending on the external perturbation.

In this paper we show the fundamental characters of the tube morphogenesis by applying an external stimulation in the form of adding nutrients to the organism. The necessary conditions under which the plasmodium is able to track the shortest path are determined.

2. Experimental methods

Various shapes of plastic films (Fuji Xerox, Japan) were prepared by cutting negative patterns of the desired shape. The plastic film was placed on the surface of a 1% plain agar plate (type s-9, Inashokuhin, Japan). An extending tip with an appropriate weight was cut from a large plasmodium in a $25 \times 35\text{-cm}^2$ culture trough and divided into small pieces. These pieces of the plasmodium were then positioned on the pattern of agar surface. A few hours later, the pieces spread and coalesced spontaneously to form a single organism extended fully on the pattern. (The plasmodium avoids spaces covered with the plastic films because of its dry surface and hence less humidity.)

Agar blocks containing 0.1 g/ml of ground oatflakes (QuakerOats, Yukijirushi, Japan) as the nutrient were set on the form and tube development was monitored by an imaging system. The experiments were carried out at 25°C.

3. Results

The basic responses of the cell shape to the food supply were determined first. A plasmodial tube newly develops from a plasmodium with sheet-like parts as they are transported onto the nutrient site (see Fig. 1a). A single thick vein is formed which connects the two initiation points

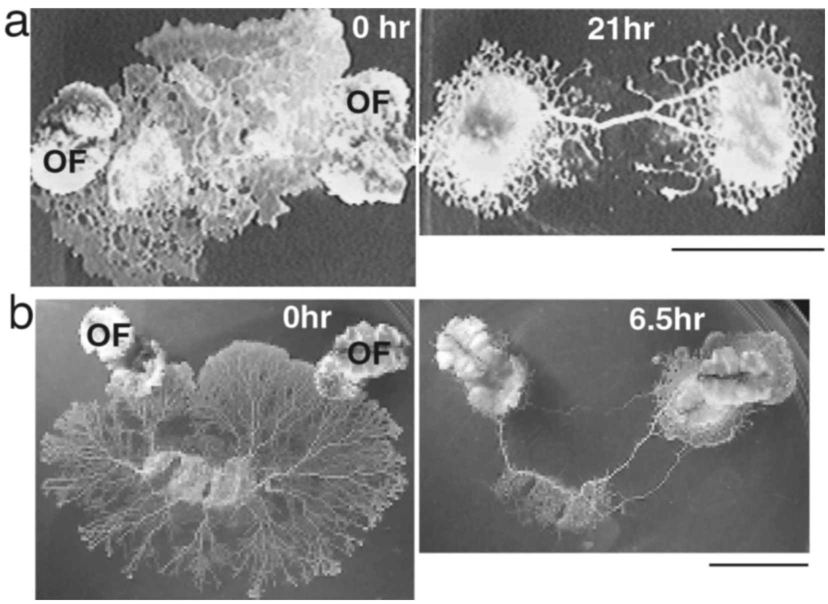

Fig. 1. Photographs of sheet-like plasmodium (a) and plasmodium with existing tube network (b) showing the tube development in response to oatflakes (OF) as food supply. Scale bars represent 1 cm.

with a length very close to the shortest path. A plasmodium with already existing tubes behaves a little differently: a single thick tube is selected and the rest including the sheet-like parts of the plasmodium disappear. The selected tube may not be the shortest connection between the two points as illustrated in Fig. 1b. Therefore, for further studies, experiments have been carried out with a sheet-like plasmodium.

A simple ring arrangement with two points was chosen to investigate the tube selection of the organism between the points (Fig. 2a). This selection has been studied by systematically varying the distance between the two food sources, characterized by the center angle of the circle, θ (Fig. 2b). For arrangements with θ = 90° and θ = 135°, the shorter tube is always selected, excluding the cases of formation of one or two tubes (see the statistics in Table 1). In cases where θ = 160°, the difference between the two possible pathways is small, the possibility of the existence of two veins increases. Thus, the plasmodium can seek the shortest path within this certain precision.

The addition of nutrients effects the structure formation, therefore, the effect of the amount of nutrient on the vein selection has also been investigated. For arrangements like Fig. 2b with θ = 90°, increasing the food amount, measured by the surface area of the agar block containing the same concentration of food, decreases the number of tubes connecting the initiation points (see Fig. 3). Two veins may exist when the food is limited. There are often no tubes with excessive food resulting in the division of the plasmodium into two individuals. In any case, the order of disappearance of tubes is such that the plasmodium of longer tubes are moved to the food sources before the plasmodium of shorter tubes. In summary, the amount of food mainly effects the time scale of the vein formation. This means that the intake of food and the maintenance of size of the organism are critical factors for the developing pattern of the connecting tubes. As shown in Fig. 3a, the number of tubes increases after approximately 20 h when the plasmodium extends from the food sites (indicated by arrows in Fig. 4) and creates connecting veins. The migration of the

Table 1
Table statistics for the path selection

Number of tubes	θ°			
	90	135	160	180
0	1	0	0	10
1 short	17	9	8	5
long	0	0	1	
2	1	1	5	7
	19	10	14	22

The number of experiments yielding 0, 1, 2 tubes connecting the food sources and at the bottom row, the total number of experiments for each arrangements are listed. The pattern formation is observed 4 h (θ = 90°, 180°) or 7 h (θ = 135°, 160°) after the addition of the food supply.

plasmodium toward the food sites lasts until the agar blocks are completely covered and soon after that the plasmodium begins to extend from the food sites. Therefore, the surface area of food is a critical factor for the tube formation under the conditions where the concentration of food is constant.

The arrangements in Fig. 5 are simpler than a maze, but have two important properties: dead ends; and multiple routes connecting two sites. Focusing on the tube morphogenesis in the dead ends, i.e. parts not connected to the food sources (AG), we have been able to show in Fig. 5 that the smaller tubes and the tubes in the dead ends shrink and disappear (Fig. 5b,c) until one or two

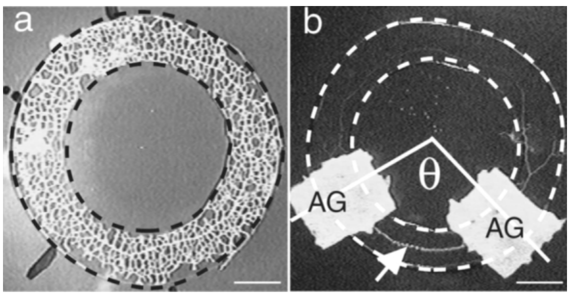

Fig. 2. Selection of the shortest connection. (a, b) Photographs of the plasmodium (10 mg wet weight) extending in a ring, bordered with dashed lines, 0 h (a) and 4 h (b) after the nutrient applied. The final path connecting the two food-sites is emphasized by a arrow in (b). Scale bars represent 5 mm. AG: the agar block containing the food as the food-source.

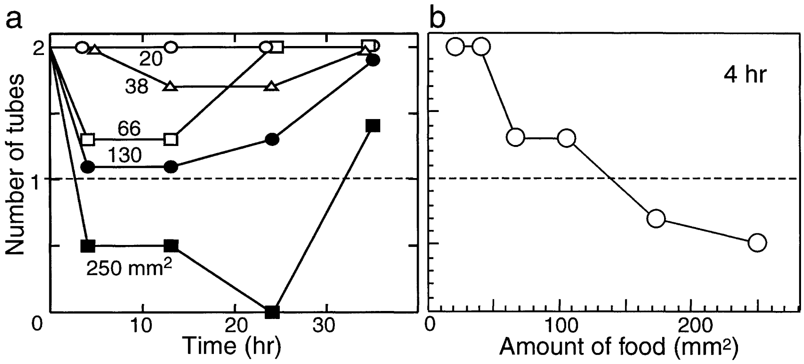

Fig. 3. Effect of food amount on the number of tubes connecting the foodsites in a ring ($\theta = 90°$). (a) Time evolution of the tube number for various amounts of the nutrient. The food quantity is measured by the surface area of the agar block containing the oatflakes. The values of the tubes are determined as the average numbers of connecting paths between two food-sites along the ring from 5–10 experiments. (b) The tube number as a function of the amount of the nutrient 4 h after the addition of the food supply.

thick veins connect the nutrient sites. The shrinking parts moved to the food-sources.

Based on these results, we have tested the path finding procedure of the organism on a more complex labyrinth shown in Fig. 6a, between two points with four possible routes $\alpha_{1,2} \times \beta_{1,2}$ (indicated by white lines in Fig. 6b). Nutrient-containing agar blocks (AG) are placed on the maze at two points. The pattern formation is summarized in Fig. 6c–e. After the shrinkage at dead ends (Fig. 6c) and the disconnection of longer connections of tube (white arrow in Fig. 6d), a single thick tube with the minimum length evolves (Fig. 6e). The paths developed by the plasmodium differ in each experiment. However, the statistics show that the occurrences of the remaining route are $\alpha_1/\alpha_2 = 0{:}14$ and $\beta_1/\beta_2 = 5{:}6$. The path through α_2 is always selected as its length is sufficiently shorter than that of α_1. The length of the route through β_1 is approximately 2% shorter than that through β_2 and the difference is within the certain precision, therefore, the plasmodium cannot differentiate between the two solutions.

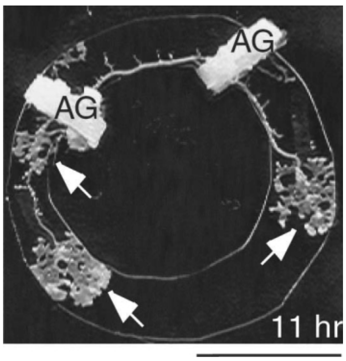

Fig. 4. Photographs of the plasmodium extending out from the food-sites 11 h after the food supply (AG). White arrows indicate the sheet-like plasmodium developed and scale bar represents 1 cm.

The plasmodial tube may extend over the plastic film of the maze, i.e. over the wall, when the amount of organism or the atmospheric humidity is very high. These extra connections across the wall occasionally lead to a path shorter than the normal shortest route (white arrow in Fig. 6f).

4. Discussion

The path finding mechanism is closely related to the contraction waves in the plasmodium. The addition of the nutrient leads to a local increase in the contraction frequency [15–17,19] which initiates wave propagation from the site of higher frequency. This induction of waves is explained by the theory of phase dynamics [18]. Such contraction waves make the tube modified, since the tube is reinforced or decayed when it is parallel or perpendicular, respectively, to the direction of

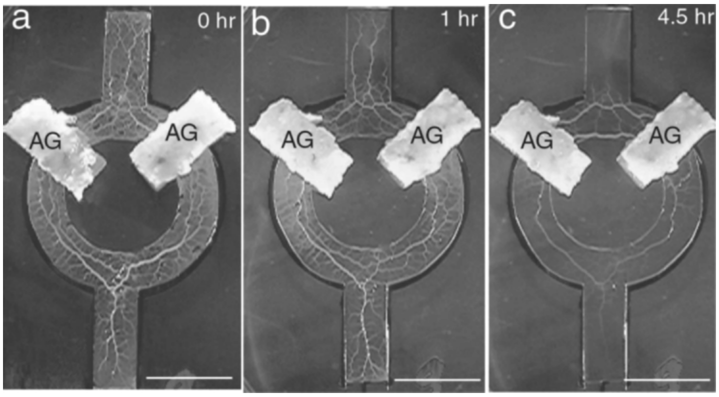

Fig. 5. Shrinking of plasmodial parts in dead-end spaces of a ring-like arrangements. (a–c) Photographs of the plasmodium 0 h (a), 1 h (b) and 4.5 h (c) after the addition of the nutrients (AC). Scale bars indicate 1 cm.

propagation of the contraction waves [14]. Therefore, effects of complex behavior of contraction waves in a maze on tube formation play a key role for path-finding in the true slime mold.

We have shown that under certain conditions the plasmodium with sheet like parts may be used to navigate the maze. Similarly, to the excitable chemical system, *Physarum* gives the minimum-length solution between two points in relation to non-linear wave propagation. However, the plasmodium occasionally tunnels through the walls in the maze establishing a route shorter than the

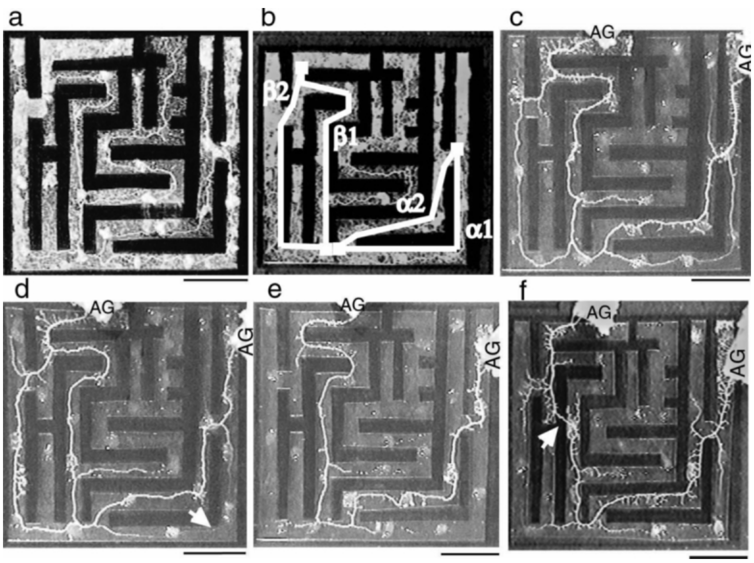

Fig. 6. Tracking the shortest path in a maze by the plasmodium. Photographs of the plasmodium (90 ± 10 mg wet weight) before [a–b(0 hr), c(4 h), d(5 h)] and after [e(8 h)] finding the shortest route. Black color corresponds to the obstacles. Scale bars indicate 1 cm. Four possible paths exist which connect the two agar blocks through α_1, or α_2 and β_1 or β_2 routes depicted in Fig. 6b. White bold lines indicate the shortest traces at α_1, (41 ± 1 mm), α_2 (33 ± 1 mm), β_1 (44 ± 1 mm) and 32 (45 ± 1 mm). (f) Photographs of the plasmodium extending over the plastic film. White arrows show the extension over the wall.

optimal path between the two points of interest by forming extra connections across the obstacles as illustrated in Fig. 6f.

We also point out the physiological aspects of the results. To maintain a single organism, the plasmodium requires all the food available in its neighborhood. The plasmodium, therefore, changes its morphology in the maze by developing one thick tube along the shortest path between the food sources to survive with the longest possible lifetime. This effective process of tracking the minimal-length solution in the maze may be considered as a cellular computation of the plasmodium by intracellular materials.

Acknowledgements

T.N. was supported by the RIKEN Institute (Frontier Research System) and by the Ministry of Education, Science and Culture in Japan (Grant-in-Aid for Scientific Research). Correspondence and requests for materials should be addressed to T.N. (e-mail: nakagaki@es.hokudai.ac.jp).

References

[1] J.A. Sepulchre, A. Babloyantz, L. Steels, in: T. Kohonen et al. (Ed.), Path finding with non-linear waves. Proceedings of the International Conference on Artificial Neural Network, Elsevier, Amsterdam, 1991, pp. 1265–1268.

[2] J.A. Sepulchre, A. Babloyantz, Motions of spiral waves in oscillatory media and in the presence of obstacles, Phys. Rev. E48 (1993) (1993) 187–195.

[3] O. Steinbock, Á. Tóth, K. Showalter, Navigating complex labyrinths — optimal paths from chemical waves, Science 267 (1995) 868–871.

[4] K. Agladze, N. Magome, R. Aliev, T. Yamaguchi, K. Yoshikawa, Finding the optimal path with the aid of chemical wave, Physica D 106 (1997) 247–254.

[5] Y. Miyake, S. Tabata, H. Murakami, M. Yano, H. Shimizu, Environment-dependent self organization of positional information field in chemotaxis of *Physarum plasmodium*, J. Theor. Biol. 178 (1996) 341–353.

[6] A. Takamatsu, K. Takahashi, M. Nagano, Y. Tsuchiya, Frequency coupling model for dynamics of responses to stimuli in plasmodium *Physarum polycephalum*, J. Phys. Soc. Jpn. 66 (1997) 1638–1646.

[7] H. Miura, M. Yano, A model of organization of size invariant positional information in taxis of *Physarurn plasmodium*, Prog. Theor. Phys. 100 (1998) 235–251.

[8] T. Nakagaki, H. Yamada, M. Ito, Reaction–diffusion–advection model for pattern formation of rhythmic contraction in a giant amoeboid cell of the *Physarum plasmodium*, J. Theor. Biol. 197 (1999) 497–506.

[9] H. Yamada, T. Nakagaki, M. Ito, Pattern formation of reaction–diffusion system with self-consistent flow in the amoeboid organism of *Physarum plasmodium*, Phys. Rev. E 59 (1999) 1009–1014.

[10] T. Nakagaki, H. Yamada, Á. Tóth, Maze-solving by an amoeboid organism, Nature 407 (2000) 470.

[11] Y. Yoshimoto, F. Matsumura, N. Kamiya, Simultaneous oscillations of Ca^{2+} efflux and tension generation in the pennealized plasmodial strand of *Physarum*, Cell Motil. 1 (1981) 433–443.

[12] Y. Yoshimoto, T. Sakai, N. Kamiya, ATP oscillation in *Physarum plasmodium*, Protoplasm 109 (1981) 159–168.

[13] S. Nakamura, Y. Yoshimoto, N. Kamiya, Oscillation in surface pH of the *Physarum polycephalum*, Proc. Jpn. Acad. 58 (1982) 270–273.

[14] T. Nakagaki, H. Yamada, T. Ueda, Interaction between cell shape and contraction pattern in the *Physarum plasmodium*, Biophys. Chem. 84 (2000) 195–204.

[15] A.C. Durham, E.B. Ridgeway, Control of chemotaxis in *Physarum polycephalum*, J. Cell Biol. 69 (1976) 218–223.

[16] K. Matsumoto, T. Ueda, Y. Kobatake, Propagation of phase wave in relation to tactic responses by the plasmodium of *Physarum polycephalum*, J. Theor. Biol. 122 (1986) 339–345.

[17] Y. Miyake, H. Tada, M. Yano, H. Shimizu, Relationship between intracellular period modulation and external environment change in *Physarum plasmodium*, Cell Struct. Dinct. 19 (1994) 363–370.

[18] Y. Kuramoto, Chemical oscillations, waves and turbulence, Springer Verlag, Berlin, 1984.

[19] K. Matsumoto, T. Ueda, Y. Kobatake, Reversal of thermotaxis with oscillatory stimulation in the plasmodiurn of *Physarum polycephalum*, J. Theor. Biol. 131 (1988) 175–182.