CONTROL OF CHEMOTAXIS IN *PHYSARUM POLYCEPHALUM*

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Cytoplasmic streaming in the myxomycete *Physarum polycephalum* is one of the best studied forms of nonmuscle movement (11, 14, 23), yet it has not hitherto been understood how the organism controls its net movement from place to place. Here we describe a control mechanism which allows the apparently ever-changing, unspecialized plasmodium of a slime mold to act as an efficient goal-seeking machine.

A *Physarum* plasmodium is effectively an enormous ameba, with a large volume of cytoplasm enclosed in one continuous external membrane. The cytoplasm streams rhythmically back and forth through a network of channels, driven by pressure gradients. External influences can superimpose a slow net movement of the whole organism on the fast shuttle streaming. Some chemicals which attract or repel plasmodia have been described (2, 3, 15, 17, 21, 26 and earlier references therein).

Previous analyses of directed movement in *Physarum* have concentrated on differences in average pressure (26). Pressure can, of course, move cytoplasm, but the crucial issue in all forms of ameboid movement is how the membrane moves. Membrane turnover and rolling like a tank-track have been ruled out for other multinucleate amebas (4, 9, 14, 28) and would be difficult to reconcile with the morphology of plasmodia.

Many large organisms move by propagating waves along their external surfaces. A similar mechanism has often been suggested to underlie ameboid movement, and can be brought up to date in terms of actin and myosin filaments (6). Time-lapse ciné films of *Physarum* show waves of alternate contraction and relaxation sweeping like peristalsis across the surface of a plasmodium (23). Therefore, we argue that the direction of movement of *Physarum* is determined by the direction in which its waves move.

A plasmodium behaves as a system of loosely coupled nonlinear oscillators. Each small piece tends to oscillate at a frequency determined by the local energy input and feedback conditions, not by a fixed resonance. It can also be entrained by neighboring oscillators. Therefore, over a substantial area the plasmodium oscillates at some average frequency. Regions of high inherent frequency establish a phase lead and initiate waves which spread towards regions of lower inherent frequency. Systems of coupled oscillators exhibiting such behavior are found in many branches of science, from economics to astronomy. Appropriate theoretical analyses have been developed for intestinal peristalsis (25) and *Dictyostelium discoideum* aggregation (20).

This logic suggests that attractant and repellent chemicals should systematically affect the inherent frequency of oscillation. While observing *Physarum* rhythms for another reason (19), we tested this prediction, but, being unaware that Rhea (18) had observed waves moving backwards from the leading edges of plasmodia, we originally expected attractants to reduce frequency. In fact the converse is true.

EXPERIMENTAL

Plasmodia of *P. polycephalum*, strain M3cV (from Joyce Mohberg of the University of Wisconsin), were grown in Petri dishes on an axenic medium (5) gelled with 2% agar. Small pieces of plasmodium were cut out and put on nonnutrient 2% agar gel, containing 1 mM NaCl and 1 mM $CaCl_2$, for the experiments reported here.

Rectangular slabs of agar gel were cooled to 18°C at one end and warmed to 34°C at the other end. Plasmodia placed on such a temperature gradient consistently migrated towards the warm end, within the temperature range 20°-30°C (Fig. 1).

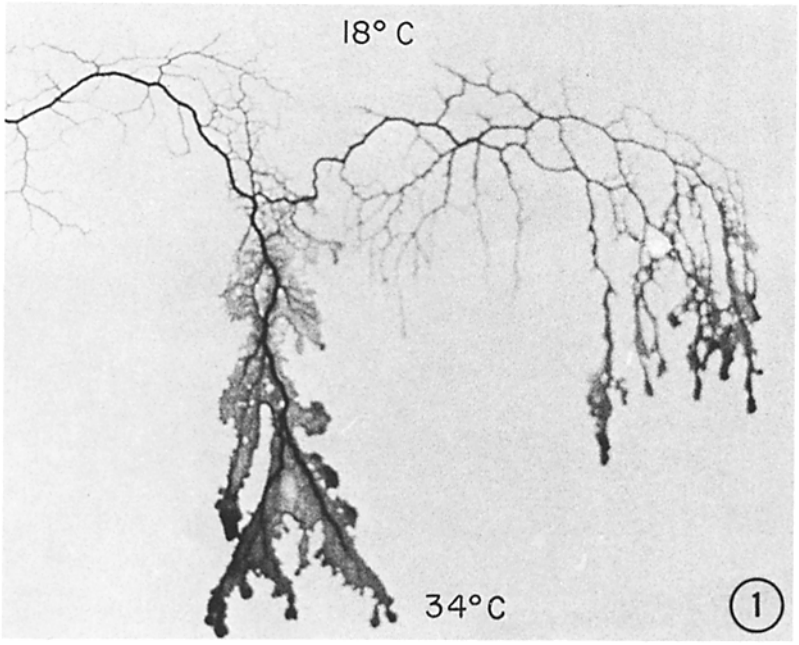

FIGURE 1 Migration of plasmodia towards warmth. Notice also the contrast between the bulbous fronts and thin tails.

The frequency of the motile oscillations of *Physarum* can be measured optically (22). We placed a piece of plasmodium about 5 mm square on agar gel in the field of view of a microscope, while a solution of 1 mM NaCl and 1 mM $CaCl_2$ flowed over it. Changes in the transmission of light in a small area of plasmodium, resulting from changes in its thickness or sideways spreading, were recorded with the aid of a photodiode set in the microscope eyepiece. The addition of 1% (= 54 mM) glucose, galactose or mannose to the solution consistently increased the frequency of motile oscillations, whereas sucrose and ribose lowered it (Fig. 2). A 1% fructose solution produced no significant change. Glucose definitely increased frequency at concentrations as low as 1 mM, but large natural frequency fluctuations prevented exact measurement of its concentration threshold.

A simple test for chemotactic repulsion was set up by putting plain agar gel on one side of a Petri dish and agar gel with a suspected repellent on the other side. Plasmodia were then placed on the dividing line and their initial directions of migration noted, until enough observations had been collected for statistical significance better than $P = 0.001$. In this assay, 10 μg/ml cycloheximide, 2 mM sodium iodoacetate and 1 mM potassium cyanide were all efficient repellents, but we made no attempt to determine their concentration thresholds. Under these conditions, 1% glucose, galactose and mannose were efficient attractants, whereas 1% sucrose and ribose were marginally repellent.

We could not entrain plasmodia to externally applied mechanical or electrical rhythms, nor find evidence for propagation of action potentials.

DISCUSSION

These results show that *Physarum* plasmodia are attracted by one agent already known to increase the frequency of their oscillations—warmth (11, 24)—and repelled by appropriate concentrations of three dissimilar chemicals already known to decrease the frequency (12, 22). Also, the frequency is increased by three sugars known to attract plasmodia, but reduced by two which repel (2). It therefore seems reasonable to induce that favorable circumstances—warmth, chemical signals indicating food, etc.—increase the frequency of alternations in streaming, while unfavorable circumstances decrease it. This idea is intuitively reasonable and in accord with such facts as the slowing in rhythm during anoxia (11) or starvation (13).

Regardless of the actual chemical mechanism for these frequency changes, but given the evident looseness of long-range coupling of oscillations in *Physarum*, it necessarily follows that mechanical waves will move away from the most favorably situated regions of a plasmodium. If these waves can move the membrane towards the front, the operation of a *Physarum* plasmodium as an effi-

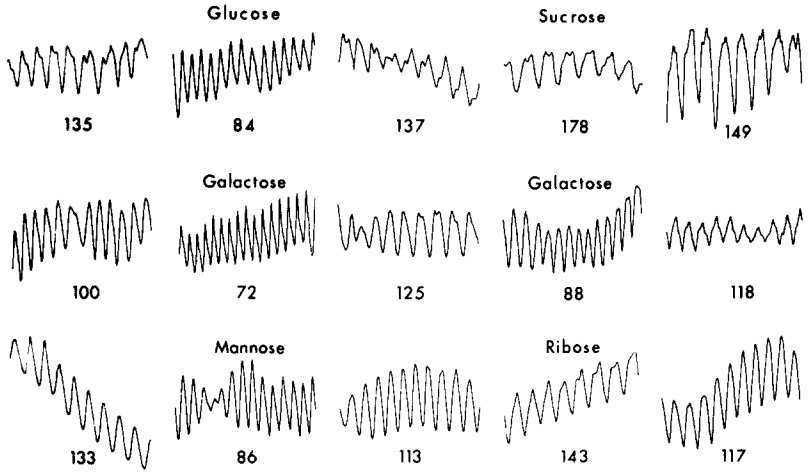

FIGURE 2 Frequency changes recorded optically. Each line shows sample recordings from one plasmodium observed over many hours, as various sugar solutions, alternating with controls, passed over them. The average period in seconds for each condition is noted underneath. Amplitude changes are not significant. Temperature 23° ± 1°C. On addition of glucose, the rhythm often stopped for several minutes as if the plasmodium was in shock. On removal of glucose, the frequency took several hours to decay to its control level.

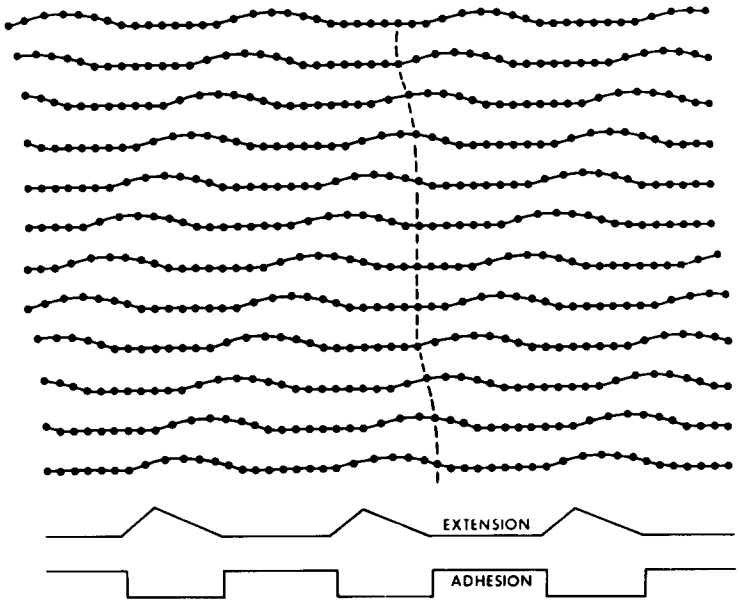

FIGURE 3 How the bottom membrane of a plasmodium could move in the opposite direction from the waves it carries. A series of lines, marked at regular intervals, represents the membrane's configuration at successive moments. Notice how a particular spot on the membrane moves from left to right. Its behavior is dictated by the phase relationship between the arbitrarily chosen membrane adhesion and extension waves moving from right to left. The leading edge may appear to ruffle, and the trailing edge may still be pulled along even if the waves die out there. In reality, the plasmodium is probably much more irregular, and points of attachment to the substrate are more localized.

cient hunter is automatic. No permanently specialized morphology is needed, and any part of the organism can rapidly become front or back at any time. Without external stimuli, movement is random, different regions establishing transitory phase leads and thereby becoming advancing fronts. Then, if any point senses food, it increases its frequency of alternation and thus draws the rest of the organism towards itself, superimposing a purposeful motion on the random element.

Fig. 3 shows one way in which a wave-bearing bottom surface might propel a plasmodium. The direction of motion is controlled by the waveforms and phase relationships of adhesion and contraction. In the diagram, they are arbitrarily chosen but basically reasonable for a surface under tension relative to the underlying substrate. Retrograde wave motion seems surprising because most of the easily visualized examples, such as a moving ruck in a carpet, or peristalsis, or a slug, involve surfaces relatively under compression, where waves and the whole body move in the same direction. However, metallurgists would recognize in Fig. 3 a form analogous to gliding edge dislocations.

Only size and regularity of rhythm distinguish this mechanism of directed movement from comparable events in a small ameba. For example, in *D. discoideum* aggregation (see 20), the wavelength is bigger than the cell. (Compare the movements of a millipede and a frog.) Of course, regular waves are only an idealized statistical way of describing movement: even in the favorable case of *Physarum*, they are highly irregular and difficult to observe on a reasonable time scale.

In a broader sense still, all mechanisms of chemotaxis are fundamentally the same, in that environmental signals are transduced by membranes into changes of frequency of alternation of ion movements. This is certainly true for sensory receptors in higher organisms (16) and the tumbling response of ciliates (7), and may well be true for the tumbling of flagellated bacteria also (1). It therefore seems reasonable to suggest that chemotaxis could be controlled by frequency in gliding prokaryotes also. Appropriate surface waves have often been postulated for gliding bacteria (10) and very likely exist in swimming spirochetes (27) also. Photometric experiments could test this prediction, but we are not familiar enough with the

bacteriological literature to assert that the necessary evidence does not exist already.

Since the movements of both cellular and acellular slime molds are controlled by traveling waves, the idea that embryological movements are similarly controlled must gain in plausibility (20). The attractiveness of this idea is that both position-sensing and movement could rely on the same process, without any need for "morphogens" if the waves are transmitted by cell–cell contacts. Furthermore, metabolic oscillations may be theoretically predicted in most cells, and Gilbert (8) claims to have observed them widely. We suggest that photometric experiments might reveal oscillations during movements of cells in developing embryos.

SUMMARY

Plasmodia migrate towards those situations which increase the frequency of their alternations in streaming, and away from those which decrease the frequency. Therefore peristalsis-like waves in *Physarum* move in the direction opposite from the net movement of the organism. This mechanism is fundamentally related to other known types of chemotaxis.

A.C.H.D. gratefully acknowledges a fellowship from the Helen Hay Whitney Foundation and facilities provided by Dr. James Spudich in part using American Cancer Society grant VC121B. E.B.R. was supported by grant no. NS10919 from the National Institutes of Health.

Received for publication 23 June 1975, and in revised form 5 December 1975.

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