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Modulation of cellular rhythm and photoavoidance by oscillatory irradiation in the *Physarum* plasmodium

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Abstract

We studied responses of cellular rhythm and light-induced movement to periodic irradiation in a unicellular amoeboid organism, the *Physarum* plasmodium. The intrinsic frequency of the contraction rhythm, which is based on biochemical oscillations, became synchronized with the frequency of periodic irradiation with light when both frequencies were close enough. In order to study the role of the synchronization in light-induced movement, periodic irradiation was applied to only part of the plasmodium. The rate of avoidance of light was modulated in the frequency band in which the synchronization occurred. The synchronization property of the contraction oscillation underlies the regulation of tactic movement in plasmodium. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cellular rhythm; Biochemical oscillation; Frequency synchronization; Amoeboid movement; Phototaxis; *Physarum*

1. Introduction

Non-linear oscillations in biological systems are of interest from the viewpoint of self-organization of physiological function. Examples include adjustment of the circadian clock, pulsation of the heart, collective luminescence of fireflies, and so on [1]. One of the key properties of non-linear oscillation is the ability to synchronize with each other, called 'synchronization', 'entrainment', 'frequency locking' or 'phase locking'. A unicellular amoeboid protozoan, the plasmodium of *Physarum polycephalum*, a true slime mold, shows non-linear oscillations of cellular contraction accompanying amoeboid movement [2,3]. This

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contraction rhythm is coupled with biochemical oscillations. Hence, oscillatory phenomena in biochemical events are easily monitored by direct observation of mechanical deformation of the cell. We studied the relationship between the property of synchronization of the cellular oscillations and the regulation of cell movement, when the plasmodium showed tactic movement in response to light. That is, the frequency modulation of the contraction oscillation and the rate of phototactic movement were observed in experiments where periodic irradiation with white light was applied to the plasmodium for various periods. From these observations, the physiological significance of the property of synchronization in cellular movement of the *Physarum* plasmodium was clarified.

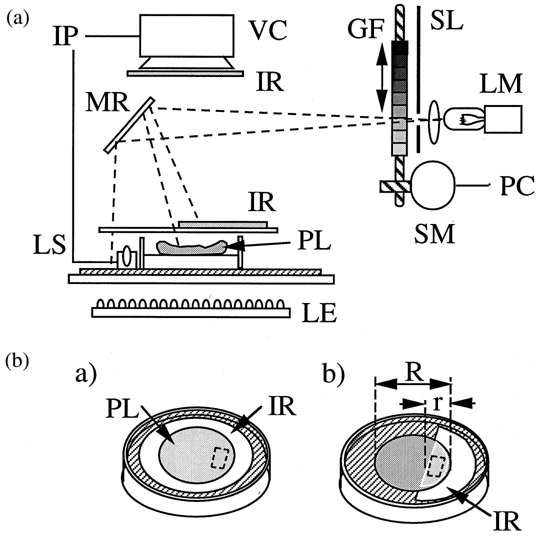

Fig. 1. (a) Schematic illustration of the experimental setup for oscillatory irradiation of the plasmodium with light. The light intensity was changed by means of a neutral density filter (GF) with continuously graded shades of optical density, moved by a shuttle slide. The sliding motion was controlled by means of a personal computer (PC) using a stepping motor (SM). The rhythmic contraction was observed by detecting the change in cellular thickness. The plasmodium (PL) was illuminated from below with LED (LE), and viewed with a video camera (VC). IP, image processing; MR, mirror; IR, cut off filter for visible light; LS, light sensor; SL, slit; LM, white cold lamp as light source. (b) Method of irradiation of the whole plasmodium (a) and a part (b) of the plasmodium. Area (r_L) of the irradiated part in the plasmodium was defined as r/R, where R is a diameter of the plasmodium and r is a width of the irradiated part with an arc-chord shape. IR and PL indicate the irradiation area and the plasmodium, respectively. Thickness oscillation was averaged over the area delineated by the dashed lines.

2. Materials and methods

2.1. Organism

The unicellular slime mold *Physarum polycephalum* (strain HU 554 × HU 560) was cultured with oatsflakes (QuakerOats, Snow Brand Co., Tokyo, Japan) on a 1% agar gel (Bacto agar, Difco Laboratory, USA) at 25°C in the dark. The extending tip (approx. 4×4 mm^2) was cut from the large plasmodium in a culture trough (25×35 cm^2) and put on an agar plate in a petri dish (9 cm in diameter). After a few hours in the dark, the organism derived from the cut portion had become extended concentrically, having a diameter of 3–4 cm, and it was used in the experiments.

2.2. Oscillatory irradiation with light, and observation of rhythmic contraction

Fig. 1A shows a schematic illustration of the experimental set-up for oscillatory irradiation of the plasmodium with light. White light from a white cold lamp (MHF-H50LR, Moritex Co., Tokyo, Japan) was employed. The intensity of the light was sinusoidally varied by means of a neutral density filter with continuously graded shades of optical density, moved by a gradual slide. The sliding motion of the filter, the speed and stroke, were controlled by means of a personal computer (type PC 9801VM, Nippon Electric Co., Tokyo, Japan). The intensity (L_I) of oscillatory irradiation was described by the equation $L_I = I_{\max} \sin(2\pi\ \omega_f t)$ where $I_{\max}$ is the maximum intensity (approx. 13 W/m^2) and ω_f is the frequency of irradiation.

Rhythmic contraction was observed by video-image analysis as described previously [4]. Briefly, rhythmic changes in thickness of the plasmodium were measured by video image analysis, since the cellular thickness varies accompanying the contraction. The plasmodium was illuminated with an infrared light emission diode (approx. 950 nm) from below, and viewed from above with a video camera. The brightness level of a pixel in the video image was related to the thickness of the plasmodium, and averaged over an appropriate area as indicated by the rectangles in Fig. 1B. The time course of brightness (I) was separated into a drifting component (DI) and an oscillatory component (ΔI) [5]. The frequency (ω) of the oscillating component was determined as the reciprocal of peak-to-peak time, and averaged over six to nine oscillations before ($\bar{\omega}_0$) and after ($\bar{\omega}$) the beginning of irradiation, because the frequency was usually fluctuating.

Light-induced movement was quantified on the basis of DI. DI is correlated with changes in anterior–posterior polarity, since the cellular thickness becomes thicker and thinner in the anterior and posterior parts, respectively. The behavior index was defined as tan θ, where the angle θ was measured counter-clockwise from the drifting component (DI) before irradiation to that after the beginning of irradiation, as shown in Fig. 4B. This index becomes positive when the plasmodium shows positive taxis or when there is a slowdown of negative taxis due to irradiation.

First, the whole plasmodium was irradiated in order to study the relationship between the intrinsic rhythm and the external oscillatory conditions (Fig. 1Ba). Then, in a second experiment, a local part of the plasmodium was irradiated (Fig. 1Bb) to examine the contribution of the property of synchronization to development of phototactic movement. The area of the irradiated part was defined as a ratio ($r_L = r/R$) of a width (r) of the irradiated part to a diameter (R) of the plasmodium.

3. Results

3.1. Synchronization of the contraction oscillation and the irradiation cycle

Fig. 2A shows the time course of changes in thickness (ΔI) before and after the beginning of periodic irradiation of the plasmodium with light, under conditions where the irradiation period was a little shorter than that of the contraction oscillation. The timing of the contraction peaks (maximum values in the time series) became synchronized with the timing of the irradiation peaks (indicated by the vertical lines in Fig. 2A). The periods of the rhythmic contraction became similar to those of the irradiation (Fig. 2B). Furthermore, the phase differences between the intrinsic and the external oscillations remained around the zero level (Fig. 2C). As mentioned above, the contraction oscillation became synchronized with the irradiation oscillation. Fig. 3 shows the relationship between the contraction frequency and the frequency of irradiation. The contraction frequency, $\bar{\omega}/\bar{\omega}_o$, was less than 1 except in the range (the hatched area in Fig. 3) where $\omega_f/\bar{\omega}_o$ was near 1. This level of $\bar{\omega}/\bar{\omega}_o$ was similar to that of stationary (not oscillatory) irradiation as shown by the dashed line in Fig. 3. On the other hand, the quantity $\bar{\omega}/\bar{\omega}_o$ was approximately proportional to $\omega_f/\bar{\omega}_o$ around $\omega_f/\bar{\omega}_o = 1$, and became more than 1. This proportional relationship means that the contraction frequency became synchronized with the frequency of irradiation. Hence, the synchronization occurred in the frequency band where the two frequencies, that of contraction and that of the irradiation, were close to each other.

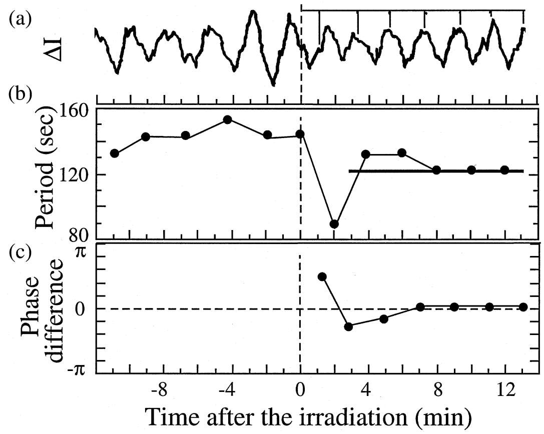

Fig. 2. Time courses of the thickness variations ΔI (a), the period (b) and the phase differences compared with the periodic irradiation (c). The vertical solid lines in A indicate the timing of application of maximum intensity irradiation. The horizontal bold line in B indicates the period of the oscillatory irradiation.

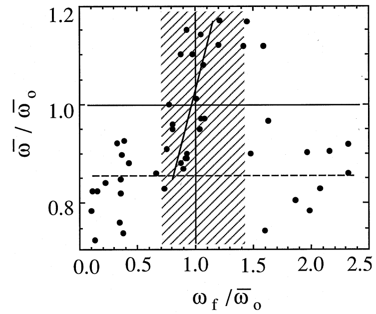

Fig. 3. Relationship between the frequency of oscillatory irradiation and the frequency of rhythmic contraction. When the two frequencies were close (as indicated by the hatched area), they became synchronized as a proportional relationship is observed along the oblique solid line with the slope of 1. The dashed line indicates the contraction period induced by stationary (not oscillatory) irradiation at an intensity equivalent to the maximum intensity of oscillatory irradiation. According to statistical analysis by least square method, a value of the slope is 0.7 with coefficient of determination, $r^2 = 0.92$. Roughly speaking, this value is similar to 1.

3.2. Modulation of the phototactic movement by periodic irradiation

Fig. 4 shows the modulation of photoavoidance occurring in response to local stimulation ($r_L = 1/4$) by oscillatory irradiation. Firstly, since the irradiation period was too short to entrain the contraction period (see the range of 6–18 min in Fig. 4A), the drifting component DI of the thickness oscillation began to decrease after the beginning of irradiation (the avoidance index was negative). That is, the irradiated part of the plasmodium responded by avoidance. Contrary to this, when the frequencies of irradiation and contraction were close enough (see after 19 min in Fig. 4A), the synchronization appeared and the avoidance behavior was modulated (Fig. 4B). Fig. 5 shows the dependency of the behavior index on the irradiation frequency. The behavior index was positive only when the frequency ratio ($\omega_f/\bar{\omega}_o$) was near 1. In the higher or lower range of irradiation frequency, such modulation of movement was not observed, and the index value was similar to that in the case of stationary (not oscillatory) irradiation indicated by the dashed line. The hatched area in Fig. 5 indicates the frequency band where the frequency synchronization occurred as shown in Fig. 3. The modulation of movement was observed only in this area. Therefore, there was a relationship between the movement modulation and the frequency synchronization.

An area of the irradiated part in the plasmodium was changed, but similar results were obtained in $r_L = 1/8–1/2$. The size and location of the rectangle to monitor the contraction rhythm were also changed, and the results as above were independent of these changes as far as the rectangle was in the irradiated part.

3.3. Some remarks on the property of synchronization of the contraction oscillation

Fig. 6 shows a series of contraction periods over a course of time when the synchronization occurred. The synchronization continued for several periods, but spontaneously disappeared (indicated by an arrow). But the synchronization ap-

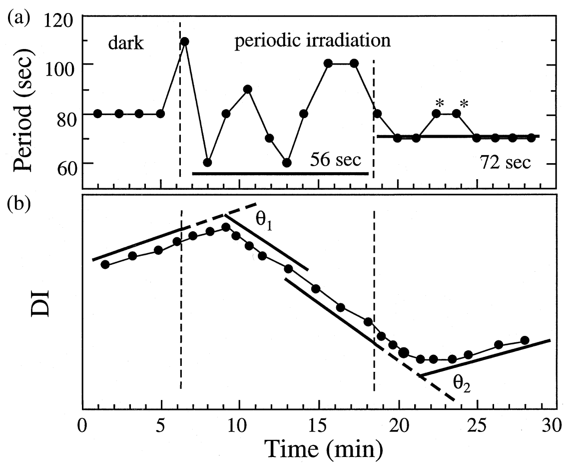

Fig. 4. Effect of periodic irradiation applied to a local part ($r_L = 1/4$) of the plasmodium. (a) Time course of the period of the thickness oscillation, ΔI. Horizontal lines indicate the period of the oscillatory irradiation. The oscillatory irradiation was applied with a period of 56 s from approximately 6–18 min, and at 72 s after 18 min. (b) Time course of the drifting component, DI. The bold lines indicate the linearly approximated baselines of DI. The angle between the baselines was measured counter-clockwise from the baseline before irradiation to that after the beginning of irradiation. So, the angles θ_1 and θ_2 were negative and positive, respectively. The behavior index was calculated as tan θ.

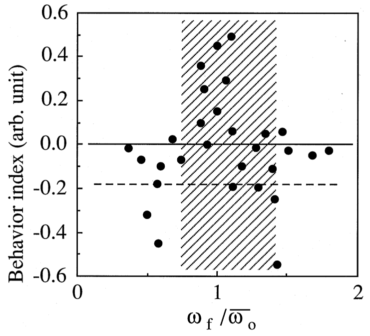

Fig. 5. Modulation of photoavoidance in association with frequency synchronization. The behavior index values are positive in the hatched area. The hatched area indicates the frequency band where the frequency synchronization occurred, corresponding to the hatched area of Fig. 3. The dashed line indicates the behavior index obtained under stationary (not oscillatory) irradiation at an intensity equivalent to the maximum intensity of the oscillatory irradiation.

peared again after the change in the irradiation period from 86 to 103 s (indicated by the arrowhead). Re-synchronization like this was sometimes observed. On the other hand, the contraction periods were not always fixed but sometimes fluctuated as indicated by the asterisks in Figs. 4 and 6. Such irregularity was responsible for the looseness of the frequency-relationship shown in Fig. 3.

4. Discussion

Is it a general occurrence in the *Physarum* plasmodium that the property of synchronization of contraction oscillation participates in regulation of tactic movement? In this study, it was assured in photomovement, and Matsumoto et al. [6] showed it in thermotaxis. As similar results were obtained using quite different kinds of stimulation, it is suggested that the answer is affirmative.

What is the physical mechanism of the modulation of tactic movement by oscillatory irradiation? The direction of tactic movement induced by stationary (not oscillatory) stimulation was found to be correlated with the direction of propagation of the contraction wave [7]. Similar wave propagation was induced by an oscillatory change in external temperature, depending on the relationship of the frequencies of the intrinsic and external oscillations, and, as the result, the direction of tactic movement was modulated [6,8]. This wave phenomenon has been reproduced by numerical simulation and theoretical analysis for some model systems of coupled non-linear oscillators [9–12]. Also, the modulation of phototactic movement may be related to such induction of a contraction wave as that mentioned above.

The synchronization and the modulation of the behavior index were limited within several cycles of contraction. This observation indicates that oscillatory irradiation with a constant period is insufficient to control the cell movement for a longer time. Something is needed to maintain the synchronization of the contraction with the irradiation. One possible factor is irregularity of the period, because the contraction oscillation sometimes reacted to a slight change in the irradiation period when de-synchronizing (Fig. 6). This is an interesting issue to be studied further.

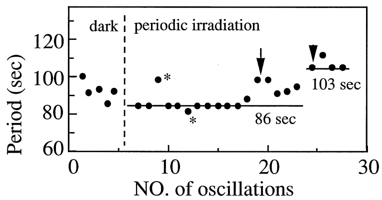

Fig. 6. Changes in the period of the thickness oscillation ΔI with respect to number of oscillations, before and after the beginning of periodic irradiation. The vertical dashed line indicates the start of irradiation. Horizontal solid lines indicate the period of irradiation. The synchronization state appeared, and disappeared later (the arrow), but appeared again after the change in irradiation period (the arrowhead).

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