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Thermotaxis and protoplasmic oscillations in *Physarum* plasmodia analysed in a novel device generating stable linear temperature gradients

R. Wolf[1,*], **J. Niemuth**[1], and **H. Sauer**[2]

[1] Biocenter, University of Würzburg, Würzburg and [2] Department of Biology, A & M University, College Station, Texas

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Summary. The application of sublethal temperature gradients offers a simple, non-invasive means for in vivo studies of thermotaxis and other temperature-dependent processes in various organisms. Development, for instance, can be dramatically desynchronized, and the resulting development gradients allow to analyze physiological interdependencies between locally separated subsystems. For this purpose a simple device has been developed, by which a stable linear gradient of 8 °C/cm is established on an inert metal sheet with the aid of Peltier elements. The effects of linear temperature gradients on fusion, growth, and migration of plasmodia of the slime mold *Physarum polycephalum* was filmed by 16 mm film time-lapse technique, and their local contraction–relaxation cycles analysed by "multistrip kymography", which represents a graphic documentation of the spatio-temporal pattern of protoplasmic movements that occur along well-defined regions within the giant cell. *Physarum* plasmodia preferentially fuse, and grow, in the range of 24–26 °C. Different parts of a single macroplasmodium can simultaneously show positive and negative thermotaxis. The contraction–relaxation cycles generating the protoplasmic shuttle streaming within the network of veins essentially depend on local temperatures and are instantaneously desynchronized by the temperature gradient. Thus they cannot be controlled by a central pacemaker or an overall electric signal. However, there is a strong tendency to locally synchronize the various oscillation frequencies present within the giant cell if temperature differences do not exceed 2 °C.

Keywords: Kymography; *Physarum*; Shuttle streaming; Temperature gradient; Thermotaxis; Time-lapse.

Introduction

Biochemical processes generally depend on temperature. As poikilothermic organisms never had to adapt to wide temperature differences within their bodies, artificially applied sublethal temperature gradients represent a unique, non-invasive means to locally retard, or accelerate, physiological processes and thus to desynchronize development (Wolf 1985, Niemuth and Wolf 1995). With the aid of temperature gradients it can be also investigated whether or not a temperature-dependent physiological process is governed by some central control system or, alternatively, by localized controls.

Temperature gradients may also serve to find out the optimum temperature range of thermotactic organisms. In many laboratories, the multinucleate macroplasmodia of *Physarum* are cultivated at 26 °C (Sauer 1982). In this paper we asked whether this temperature is actually preferred by growing plasmodia. In addition, the effect of a temperature gradient on the oscillating contractions of the plasmodium was investigated, and we asked whether there might be some central pacemaker that governs the characteristic contraction–relaxation cycle (Achenbach and Wohlfarth-Bottermann 1980a, b). For this purpose we have analyzed the well known contraction–relaxation oscillations in the ectoplasm of the plasmodium which generate the shuttle streaming. These oscillations can be monitored as the changes in transparency of the plasmodium by time-lapse cinematography and analyzed by kymography. Film analysis has been successfully used to study the degree of synchrony of contraction cycles in veins throughout the plasmodium by

* Correspondence and reprints: Biozentrum, Universität Würzburg, Am Hubland, D-97074 Würzburg, Federal Republic of Germany.

Grebecki and Cieslawska (1978a, b). Finally we ask whether there is a temperature optimum for the fusion process of microplasmodia.

For these experiments the temperature gradient apparatus described by Wolf and Wolf (1974) has been substantially improved, thus allowing to maintain the gradient over virtually any time required. Recently our apparatus also served to analyze thermotactic orientation of parasitic trematodes within their host's bodies (Haas 1994, Haas et al. 1987).

Material and methods

Apparatus to generate stable temperature gradients

Steep linear temperature gradients of 8–80 °C/mm (or K/mm) had been formerly established within a 1 mm thick agar block in the transparent temperature gradient apparatus designed by Wolf and Wolf (1974), which could be attached to the stage of any inverted microscope for monitoring temperature effects on small organisms embedded in the agar. Recently, this apparatus was improved by installing two 19 W Peltier elements which served as heat pumps (Niemuth and Wolf 1995). The temperatures generated at the two sides of the Peltier elements were transmitted by 5 mm copper strips, which served as "temperature poles" for the temperature gradient established between them (Fig. 1).

A heat-conducting support with sufficient thermal conductivity is required to maintain stable linear temperature gradients within large organisms. As the heat-conducting parts in our apparatus were not insulated, heat transport was not restricted to the direction of decreasing temperature within the gradient, but there was a considerable loss by thermal radiation and by heat exchange with the surrounding air. If the support, along which the temperature gradient is established, is conducting heat very well, there will only result a flat, yet very precise, linear gradient. If heat conductivity is poor, however, the arising gradient will be steep, but no more linear. To optimize the conditions we connected the "temperature poles" generated by the Peltier elements through a 0.6 mm metal sheet of 21.5 × 32 mm size made of copper or silver (Fig. 1). Within that metal sheet the temperature dropped linearly as measured with a calibrated contact thermometer.

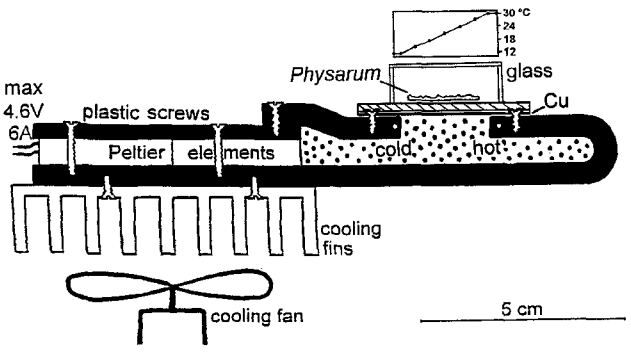


Fig. 1. Apparatus for establishing a stable linear temperature gradient within an intact macroplasmodium of *Physarum*, which allows for simultaneous monitoring of behavior by filming. *Cu* Copper sheet connecting the "temperature poles"; dotted area, sheet of expanded plastics for thermal insulation; striped area, nutrient agar. Sideview, drawn to scale

Air conditioning and circulation kept the room temperature at 26 ± 0.2 °C. Throughout the experiments the stability of the temperature gradient was guaranteed by continuous registration of the temperatures at the "temperature poles". 3 min after the Peltier elements had been switched on, a stable gradient of 7 ° C/cm ranging from 13 to 30 °C was established over the 21.5 mm distance within the layer of nutrient agar (see below), which covered the 0.6 mm copper sheet between the "temperature poles". Deviations from linearity amounted to 0.2 °C maximum, as measured with a calibrated 0.08 mm copper/constantan thermocouple.

In order not to interfere with the gradient, heat conductivity of the investigated organism must be considerably lower than that of the heat-conducting support, which was the case in our apparatus. As the mass of the protoplasm streaming in the veins is tiny (20–21.5% the total cell mass; Grębecki and Cieslawska 1978a) compared to the rest of the plasmodium plus the agar support, the temperature gradient remained virtually undisturbed by the shuttle streaming.

Photographic technique used for documentation of Physarum plasmodia

Physarum polycephalum (strain CL) was grown as a shaken suspension of microplasmodia in axenic medium (Daniel and Rusch 1961, Daniel and Baldwin 1964). Macroplasmodia were obtained as usual by allowing microplasmodia, which had been harvested during their exponential growth phase 24 h after inoculation into fresh medium, to fuse on a 2 mm layer of 4% agar made with axenic growth medium (Daniel and Baldwin 1964) as a nutrient substratum which also served to support normal growth.

To monitor the effects of temperature gradients on *Physarum* plasmodia and their contraction–relaxation cycles, developing micro- and macroplasmodia were continuously filmed at 15–300 frames/h with Kodak technical pan. 15–300 frames/h were taken with a Bolex 16 mm camera equipped with a Zeiss Luminar lens (f = 63 mm). A single 30 W low-voltage lamp was used to provide an oblique dark field epi-illumination switched on for 2 s only during each frame exposure, while heat radiation was virtually eliminated by appropriate filtering. To minimize light reflection from the agar support the metal sheet had been covered with black paint. The agar layer also served to avoid uncontrolled flow of heated liquid between the temperature poles. To protect the agar against desiccation the air space above it was isolated by a glass cover (Fig. 1) provided with anti-condensation medium, which is normally used for spectacles.

Indirect strip microkymography of the contraction–relaxation cycles within Physarum macroplasmodia

Kymograms essentially represent a graph showing every change of brightness, or visible movements that occur along a well-defined, freely selectable spatial axis of the filmed organisms (Kamiya 1959). Kymograms are made by projecting a (time-lapse) film through a narrow slit onto photographic paper mounted on the rotating drum of a kymograph (Wolf 1976, Wolf and Nuss 1976, Grębecki and Moczori 1978). This technique was modified to record motions that occurred simultaneously at different sites of a single macroplasmodium. Synchronic records of contraction–relaxation cycles at different sites of a plasmodium had been done by Grębecki and Cieslawska (1978b) by a photocell mounted at different sites of the image during repeated projections of the same film. In our approach the running film was projected onto a black metal sheet, which contained 35 slits

of 4.5 × 0.6 mm arranged in a regular pattern (Fig. 5), each of them giving rise to a single, slim strip kymogram. Although the slits were designed to cover the range of local temperatures all over the macroplasmodium, multistrip kymography is not an optimum technique. The local oscillation did not show up in every slim strip because the positions of the slits could not be adapted to veins locally present in the image, or to their orientation. Nevertheless the oscillation patterns over the plasmodia were well captured in the resulting multistrip kymograms (Fig. 6).

The kymographic record of movements always started at the same time but at different regions of the filmed object. To calibrate the time scale, every onehundredth frame of the time-lapse film was marked in black. The resulting white bars on the (negative) multistrip kymogram served to rearrange the single strip kymograms in order to generate a unitary time scale. For this purpose the resulting multistrip kymogram was cut into small strips parallel to the time axis, and the strips were rearranged so that all individual kymograms started at the origin of the abscissa (Fig. 6). Processes that took place simultaneously at diverse regions of a macroplasmodium thus were represented at identical values of the abscissa.

The cyclic contraction of veins in a macroplasmodium shows up in subtle changes in brightness, due to changes of local light reflection, within each of the single kymograms. The pattern of dark and bright stripes recorded in diverse regions of the macroplasmodium thus mirrors the local contraction–relaxation cycles in the space–time diagram.

Results

Growth of Physarum macroplasmodia on nutrient agar

Macroplasmodia represent a vigorous vegetative stage within the life cycle of *Physarum*. They can migrate up to 5 cm/h over a moist substratum while establishing a distinct anterior–posterior polarity. The posterior lobe mainly consists of a network of strands which extend and fuse into a rather homogeneous system of veins towards the fan-like anterior region with its characteristic leading ridge. Within the strands and the veins a vivid rhythmical shuttle streaming provides for an exchange of cytoplasm and millions of nuclei within the giant cell. The subcellular constituents are shifted at speeds up to 1.3 mm/s by the shuttle streaming, which usually changes its direction every 60 to 75 s, i.e., the normal cycle length at 22 °C is 120–150 s (Achenbach and Wohlfarth-Bottermann 1980a, b).

In the absence of a temperature gradient, plasmodia expanding under optimal growth conditions on the agar layer of our apparatus generally did not develop an anterior–posterior polarity, but rather extended in all directions (Fig. 2). Such spreading as a flat disk is typical for balanced growth of macroplasmodia on solid support and is utilized for cell cycle studies with homogeneous starting material (Sauer 1982). In this control experiment, which was performed twice, plasmodia grew out continuously over 48 h, with the exception of 10 min interruptions which took place every 9.2–9.5 h. Presumably, the synchronous mitoses, which reportedly occur about every 10 h under these conditions, correlate with the transient growth arrest (Miller et al. 1968).

Thermotaxis of Physarum macroplasmodia

In 25 experiments the migration of one to three macroplasmodia, which had been cultivated 8–24 h on nutrient agar, was filmed under the influence of a

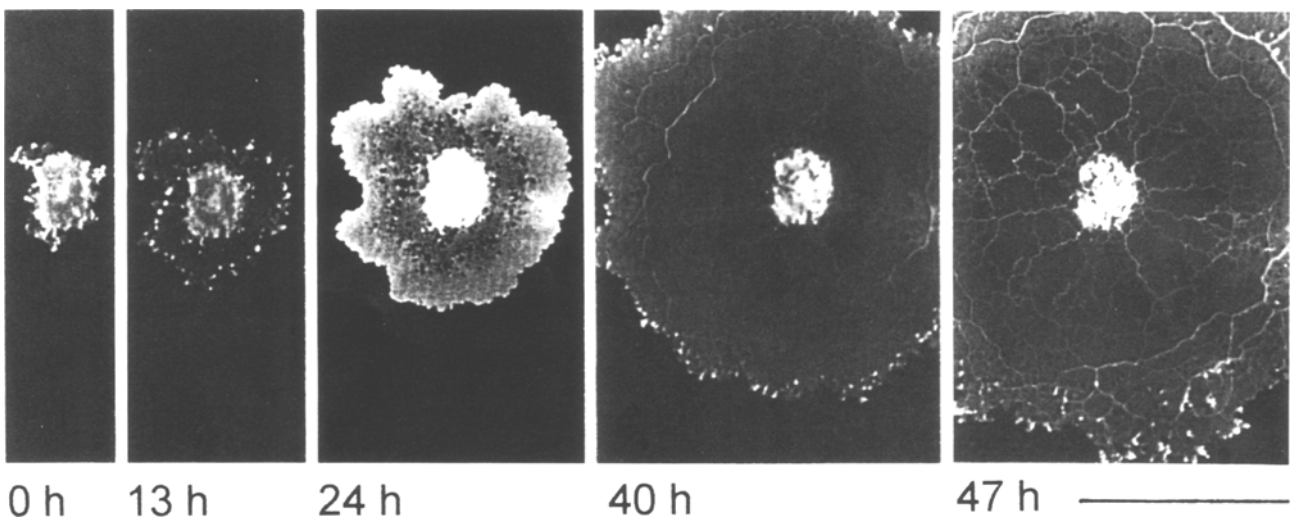
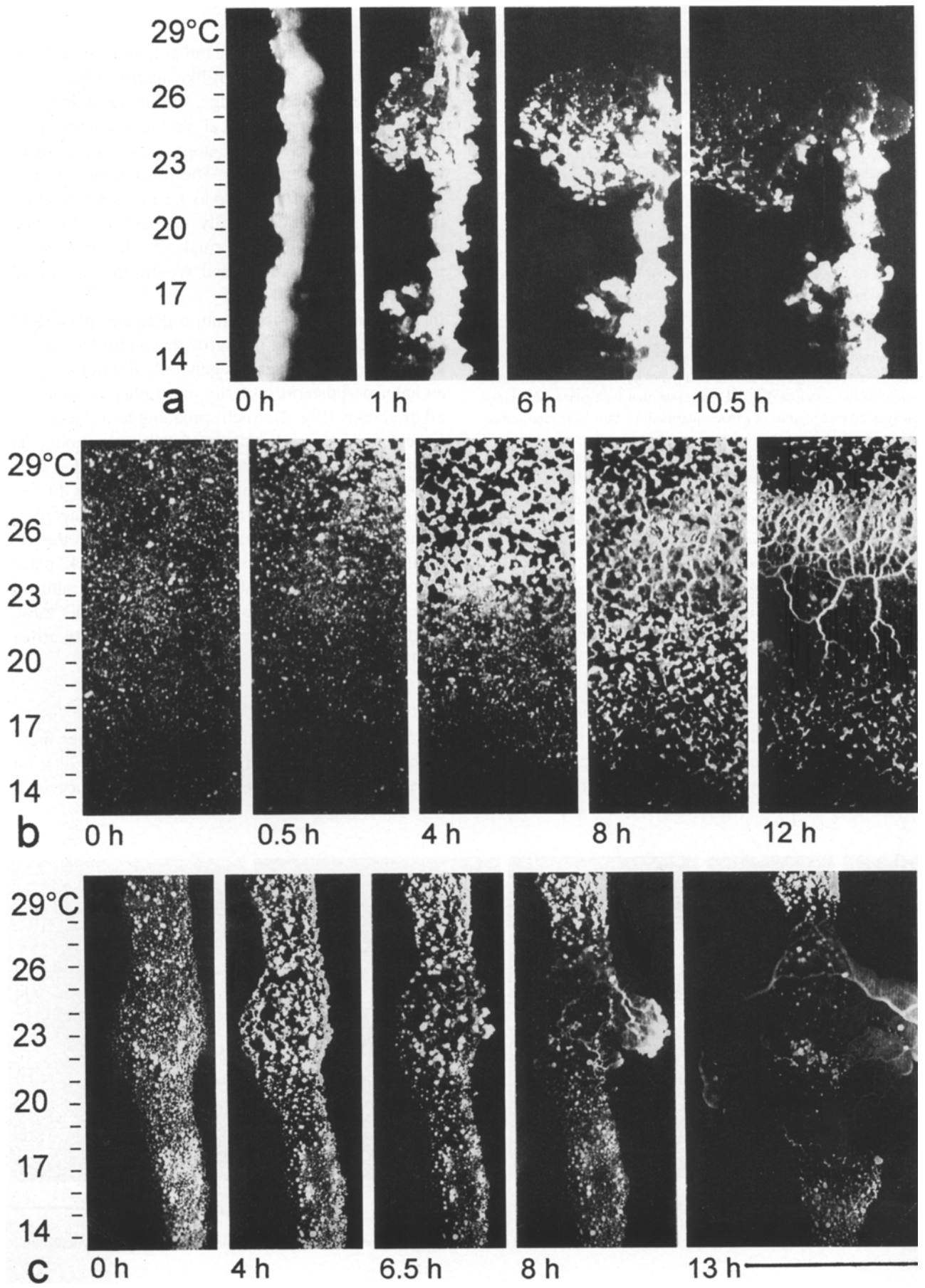


Fig. 2. Normal macroplasmodium growing on a layer of nutrient agar in the temperature gradient apparatus, but with the gradient switched off. Bar: 1 cm


29°C
26
23
20
17
14
a
0 h
1 h
6 h
10.5 h
29°C
26
23
20
17
14
b
0 h
0.5 h
4 h
8 h
12 h
29°C
26
23
20
17
14
c
0 h
4 h
6.5 h
8 h
13 h

stable gradient of 7 °C/cm. In each case plasmodia showed a characteristic thermotactic behavior. When a small macroplasmodium of 2–3 mm size was positioned between 13 and 24 °C, pseudopodia developed in the direction towards the warmer pole, and the whole organism started to move in that direction. However, it did not enter regions warmer than 26–27 °C but rather grew sidewards, i.e., in isothermic directions, thus forming a sharp linear front towards the warmer regions. Maximum growth took place between 26 and 24 °C. However, plasmodia also were observed to migrate towards a region of somewhat lower temperature, between 22 and 26 °C, and to grow out from there. Small macroplasmodia, which had been transferred to various regions between 16 and 28 °C within the gradient, grew towards the optimum region where the temperature of the agar was between 24 and 26 °C. In this region they tended to fuse spontaneously, thus forming a syncytium. Neither growth nor migration could be observed in plasmodia which were originally positioned within regions above 29 °C, or lower than 15 °C. When a macroplasmodium had been arranged to form a strand ranging all over the temperature gradient from 13 to 30 °C, it started to grow sidewards, i.e., in an isothermic direction, within the 23–27 °C range (Fig. 3 a).

Fusion of Physarum microplasmodia within the temperature gradient

At 26 °C a cluster of 1000–2000 *Physarum* microplasmodia transferred onto a layer of nutrient agar readily fused into a single macroplasmodium within 1 h. A different pattern, however, was observed in microplasmodia exposed to a temperature gradient. In a series of five experiments 1000–2000 microplasmodia harvested from a shaken suspension during exponential growth were transferred on the agar layer and submitted to a temperature gradient of 7 °C/cm. Under these conditions the fusion process started somewhere between the 27 and 29 °C isotherm and proceeded from there as a unidirectional fusion wave into the colder regions at a rate of 0.8–1.4 cm/h (Fig. 4). After 4 h the fused protoplasm was concentrated between 24 and 27 °C and had established a pattern of veins (Fig. 3 b). During the following 18 h the protoplasm slowly moved to and fro between the 20 and 27 °C isotherm, thereby changing its migration direction every 5–8 h.

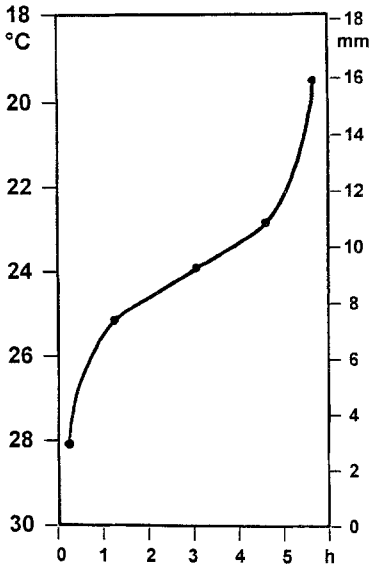


Fig. 4. Spatiotemporal pattern of the fusion wave observed within microplasmodia distributed all over the temperature gradient (cf. Fig. 3 b)

When 1000–2000 microplasmodia were arranged as a narrow strip between the temperature poles, fusion again occurred almost simultaneously between 27 and 29 °C and proceeded from there into the colder regions. After 6–7 h the first protoplasmic fans of the resulting macroplasmodium grew out somewhere between the 22 and 25 °C isotherm (Fig. 3 c).

Desynchronization of the contraction–relaxation cycle by the temperature gradient

Temperature dependence of the contraction–relaxation cycle was filmed in a total of 12 macroplasmodia of 16–20 mm diameter. In each of these experiments two small supplementary macroplasmodia of 3–4 mm diameter were added, one of them located close to warm pole, and the other one close to the cold pole of the forthcoming temperature gradient. The plasmodia were first grown on a 2 mm layer of nutrient agar. When the macroplasmodia had reached their appropriate size they were transferred, together with their

Fig. 3. a Protoplasmic strand of a *Physarum* macroplasmodium exposed to a linear temperature gradient. Migrating fans are seen to grow out within the temperature range of 24–26 °C. **b** Fusion wave of microplasmodia distributed all over the temperature gradient. A net of veins starts to develop 8 h after the beginning of the experiment. **c** Fusion process in a strand of microplasmodia, with the first protoplasmic fan growing out at 23–25 °C after 8 h. Bar: 1 cm

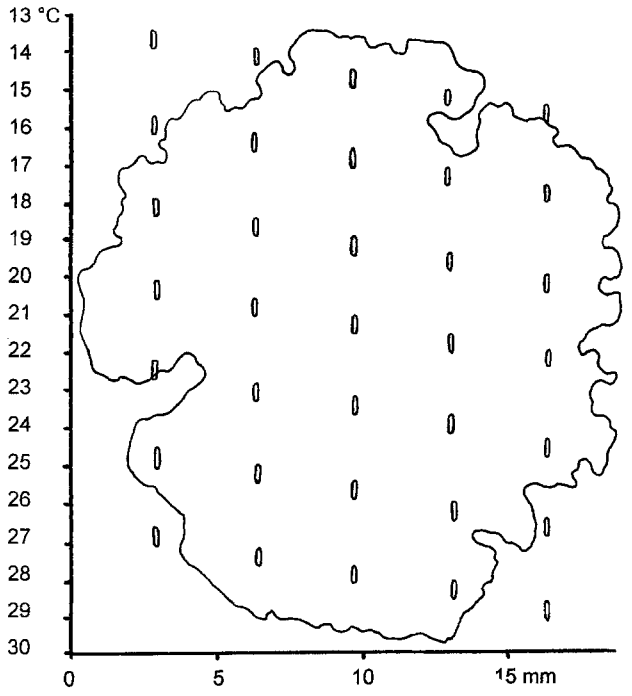


Fig. 5. The multislit metal sheet used to record the local contraction–relaxation cycle within a plasmodium documented in a time-lapse film. Abscissa: spatial axis of the record. Ordinate: range of local temperatures in a macroplasmodium, the shape of which is indicated by dotted lines

substratum, onto the 0.6 mm copper sheet with the temperature gradient still switched off, and filmed at 5 frames/min. After the transfer procedure the contraction–relaxation cycle generally had been interrupted, but was readily resumed within 5–10 min. 30 min after the transfer the temperature gradient of 7 °C/cm was established, and its effects were continuously monitored on time-lapse film.

We analyzed the contraction–relaxation cycle in four experiments, where all three macroplasmodia originally happened to oscillate at rather similar frequencies, with cycle lengths ranging from 58 to 65 s. The result of one of these experiments is shown in Figs. 6 and 7. A characteristic desynchronization of the protoplasmic oscillations was observed as soon as the temperature gradient had been established. As indicated by the rhythmical changes in brightness of the veins, the frequency of the contraction–relaxation cycles initially decreased in those parts of the same giant cell which were colder than 26 °C, but simultaneously increased in the warmer regions. Specifically, between 27.5 and 29 °C the cycle length initially decreased to 47–53 s during the first 40 min, but then again slightly increased to 50–58 s. Simultaneously, the cycle length initially increased dramatically between 19 and 25 °C, but then increased again (Fig. 7). In the region below 17–18 °C the oscillations stopped completely 10–20 min after the temperature gradient had been established, and were not resumed before further 30–50 min. Only after a total of 80–90 min the contraction–relaxation cycles had stabilized and displayed a stable frequency gradient as a response to the stable temperature gradient constantly present.

As shown in Fig. 7, different domains of a single macroplasmodium, which were exposed to different local temperatures (26 and 28 °C), oscillated at increasingly different frequencies during the first 40 min after the gradient had been established (cycle lengths: 60 and 49 s, respectively). Thereafter these domains virtually resynchronized their contraction–relaxation cycles over a period of half an hour (final cycle length: 51 s). Remarkably, the oscillation frequency initially decreased by 16–25% during the first 40 min, but then increased again and eventually was the same as at the beginning of the temperature gradient. Finally, in the 26 °C region the frequency of the contraction–relaxation cycle was approximately 20% higher than at the beginning of the gradient, even though it occurred in an isothermic region.

The contraction–relaxation cycles in the supplementary small plasmodia, which developed within a narrow temperature range of the gradient, differed significantly from the final frequencies measured in isothermic regions of large macroplasmodia (Fig. 7). On average, small plasmodia oscillated 14% faster than the isothermic regions of large plasmodia when situated close to the warm pole of the gradient (between 27 and 29 °C), but 11% slower when growing in the cold region (between 15 and 18 °C).

When the temperature gradient had been switched off after it had acted on the plasmodia for 2–3 h, temperatures within the giant cell adjusted to ambient temperature (26 °C) within 2 min. As a consequence, the contraction–relaxation cycles were slowly resynchronized throughout the protoplasm within 16 to 30 min.

Discussion

General aspects of developmental desynchronization by a temperature gradient

Being exposed to a temperature gradient, a poikilothermic organism has to adapt to an entirely novel situation. Recently, temperature gradients were applied to induce developmental gradients which served as a

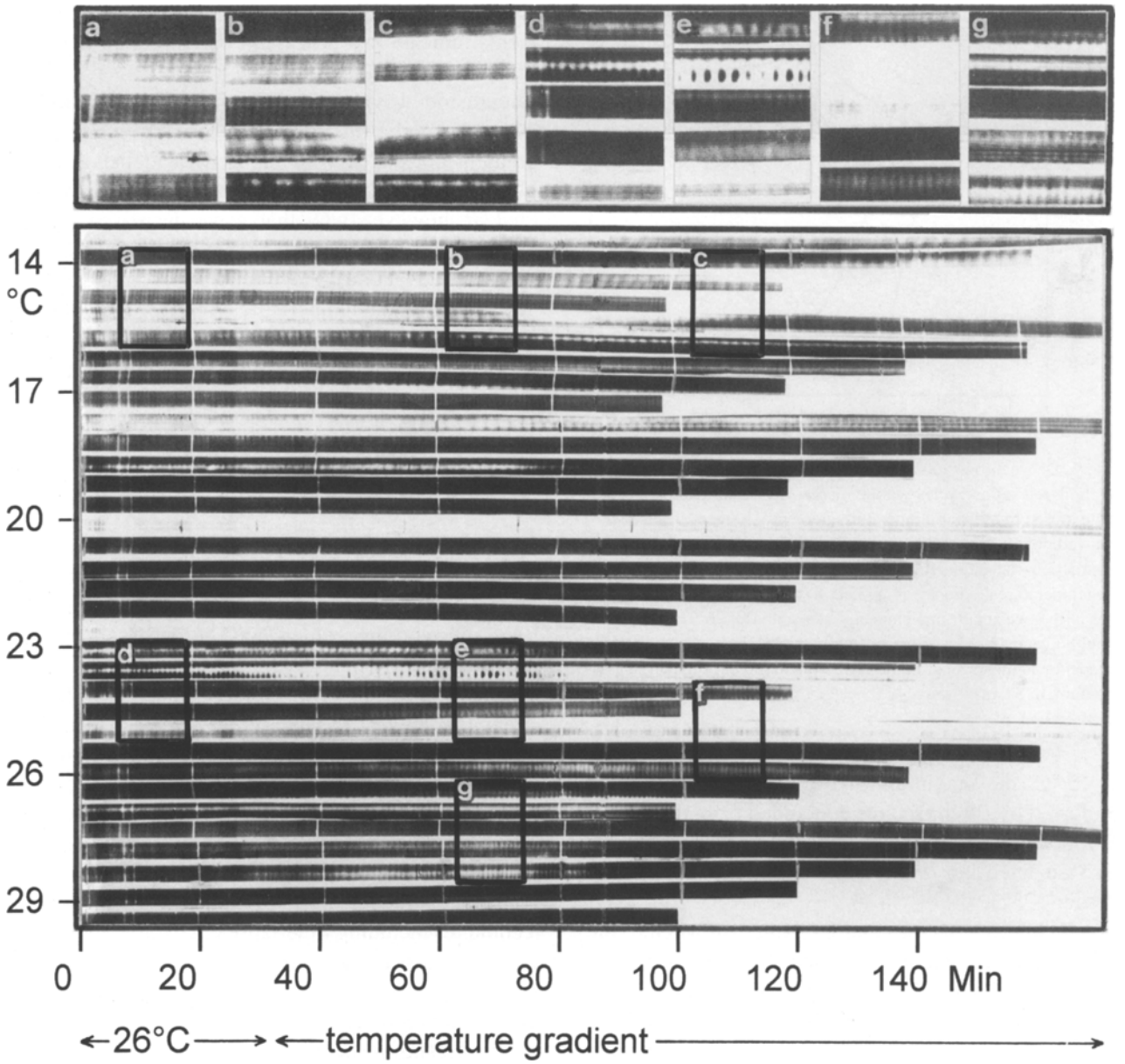


Fig. 6. Multistrip kymogram of the contraction–relaxation cycles within a *Physarum* macroplasmodium with the oscillation desynchronized by a linear temperature gradient of 7 °C/cm. The original kymogram has been cut into strips which were rearranged to obtain a unitary time scale. The frequency of the contraction–relaxation cycle can be read off from the local changes in brightness, which appear as a regular stripe pattern in many of the small single kymograms. **a–g** Details from various regions of the multistrip kymogram

valuable tool to investigate physiological interdependencies, for example, between locally separated subsystems inside early insect embryos. There is a remarkable capacity in insects to regulate, despite development being widely perturbed, a normal embryo. The early development of the parasitic hymenopteron, *Pimpla turionella*, has been dramatically desynchronized, with nuclear division cycles being out of phase by seven generations. While gastrulation had started in the warm region, midblastula transition and cellularization were in progress in the middle of the egg, whereas intravitelline nuclear multiplication occurred at the cold pole by rapid, and still biphasic, cell cycles comprising mitosis and S-phase only (Niemuth and Wolf 1995).

In the present study temperature perturbations were introduced in a single cell. From the behavior of *Physarum* macroplasmodia within a temperature gradient, the main characteristics of their temperature-dependence have been derived: the growth minimum

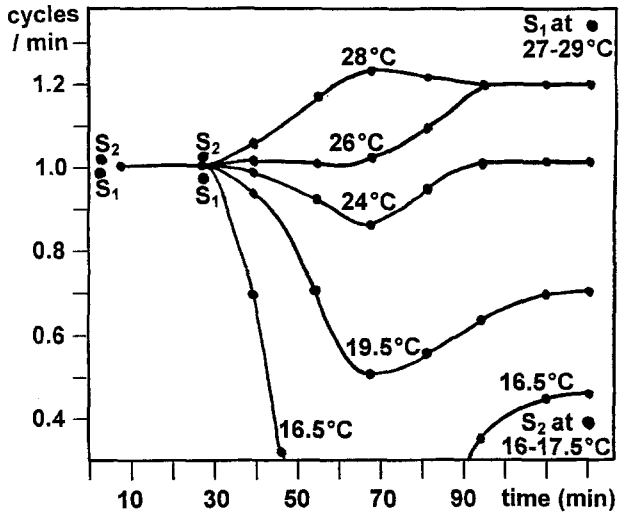


Fig. 7. Desynchronization and local resynchronization of the contraction–relaxation cycles within a large *Physarum* macroplasmodium exposed to a linear temperature gradient (see text). The plasmodia were left at 26° for 30 min to allow the establishment of a stable contraction–relaxation rhythm at a constant temperature, and then the temperature gradient was applied. S_1 and S_2, contraction frequencies of the two small supplementary plasmodia, at 26 °C (left) and at 27–29 °C and 16–17.5 °C, respectively (right). In S_1 the contraction frequency is higher than in the isothermic domain of the large plasmodium, in S_2 it is lower

at 15 °C, the maximum at 29 °C, and the optimum preferred by *Physarum* (as concluded from the migration behavior) at 26 °C. This is actually the temperature selected for cultivation in many laboratories (Sauer 1982).

In our experiments we cannot distinguish whether the observed thermotactic movements of the macroplasmodia actually resulted from preferential growth, or exclusively from migration. It has previously been shown that it is impossible to distinguish between growth and pure migration in *Physarum* (Knowles and Carlile 1978).

Plasmodia growing towards their temperature optimum from colder regions tend to move "too far". They generally overshoot their optimum and reverse migration direction close to the 27 °C isotherm, i.e., at only one degree above their optimum. Similarly, when approaching the optimum from warmer regions, the point of return is at about 23 °C which is 3 °C below optimum. Positive and negative thermotaxis thus forces the plasmodium migrate to and fro within 23–27 °C range of the gradient. This observation confirms and extends the results of Tso and Mansour (1975) who found out that a 3 °C interval is the minimal thermal gradient upon which a thermotactic response can be observed in *Physarum*. Our experiments indicate that at least in one direction the level of discrimination might be only 1 °C.

The fusion wave observed within a population of microplasmodia distributed all over the temperature gradient correlates with the general dependency of physiological processes on temperature. It remains unclear, however, why that wave decreased significantly between 25 and 23 °C, but then was accelerated while advancing into the colder regions (Fig. 4). These suboptimal temperatures for growth may represent a range that is critical for fusion. This may also explain why the fusion wave is asymmetrical and propagates only from warm to cold.

Effects of the temperature gradient on the contraction–relaxation cycles

The shuttle streaming, which is generated by the vein's contraction–relaxation cycles, is a unique feature of plasmodial slime moulds. It serves to homogenize a large plasmodium, which may be necessary for the precise natural synchrony of nuclear divisions.

Time correlation of vein contraction and protoplasmic streaming cycles was analyzed in many investigations. Oscillations of streaming direction do not simply reflect the contraction–relaxation rhythm. Contraction promotes outflow of endoplasm, but the inflow favors expansion. Previous authors detected different phase shifts between these two oscillatory phenomena. Moreover, utilizing numerous non-invasive techniques including time-lapse cinematography, photometry and strip kymograhy, it had been demonstrated that synchronization of contractions in a plasmodium is much more precise than the synchronization of the streaming (Sachsenmaier and Blessing 1973, Baranowski 1976, Mustatich and Ware 1977, Grębecki and Moczori 1978, Grębecki and Kolodziejczyk 1983, Kolodziejczyk and Grębecki 1982).

Recently, the relationship between oscillation frequencies and temperature was investigated by Halvorsrud et al. (1995b) who employed a very sensitive technique to a single plasmodium over a period of several days. Electrical cell-substrate impedance sensing (ECIS) allows to monitor changes in the distance between the cell surface and an electrode, down to 0.1 nm. In this procedure a constant AC voltage of 1 V at 4 kHz, in series with a resistor of 1 MΩ, is applied to the plasmodium, and fluctuations of the impedance are measured and analysed. It is assumed that the changes of the impedance reflect normal con-

traction–relaxation cycles of the whole plasmodium, as no physiological effect by a 1 μA alternating current on the plasmodium has been detected. Beginning at 30 °C, the temperature was altered in steps of 1 °C down to 1 °C and then back up again to 30 °C. As expected, there was a general decrease in the oscillation frequency correlated with a lower temperature. In the physiological range the period lasted about 2 min at 16 °C and 1.25 min at 28 °C. These are average values, and individual plasmodia may have slightly (±20 s) different rhythms. Similar average values (2.31 min at 14 °C and 1.26 min at 30 °C) were measured by Wohlfahrt-Bottermann (1977). In our experiments the small separate plasmodia at about 16.5 °C and 28 °C, respectively, and the single larger plasmodium that was exposed to the temperature gradient of that range, reveal a similar trend, although the difference in the period is greater (about three-fold, ranging from 2.5 min at 16 °C to 0.8 min at 28 °C; Fig. 7). We suspect that culture conditions and/or the age of the stock cultures may be responsible for the differences between the periods measured in different laboratories.

Remarkably, at unphysiological temperatures, below 10–15 °C, where previous studies (Kamyia 1959, Wohlfahrt-Bottermann 1977) had not described oscillations and the present study has revealed neither oscillations nor outgrowth or spreading of a plasmodium, impedance fluctuations could still be detected down to 1 °C. At that temperature the period lasted over 13 min. It is probable that the oscillations of the plasmodium at unphysiological temperatures have been overlooked by the traditional methods and could only be discovered by the highly sensitive ECIS technique. However, it cannot be excluded that the low alternating currency passed through the cell may have provoked a physiological effect and somehow sensitized the plasmodium to oscillate. This possibility can only be addressed when the contraction–relaxation cycles and the shuttle streaming of a single plasmodium will have been analyzed simultaneously by ECIS and another non-invasive procedure.

In normal plasmodia the contraction–relaxation cycle occurs at an almost constant frequency, and the oscillation is approximately in phase throughout the cell, with the exception of a small frontal region. *Physarum* plasmodia thus are considered "imperfect synchronized monorhythmic contractile systems" (Grębecki and Cieslawska 1978b; Hülsmann and Wohlfarth-Bottermann 1978a, b; Grębecki 1979). Despite decades of analyses (Stockem and Brix 1994, Sauer 1982) the identity of the oscillator has remained elusive. It is clear, however, that the shuttle streaming is a passive flow of endoplasm driven by the contraction cycles of the ectoplasm. The rhythm is generated by actomyosin contraction and relaxation cycles. The actomyosin cortex generates motive force which is transformed into hydraulic pressure gradients that result in protoplasmic streaming via gel–sol transitions. Intracellular Ca^{++} is involved in both microfilament assembly and contractile activities. Free Ca^{++} levels control myosin ATPase activity through the Ca^{++}-binding myosin light chain and via myosin and actin kinases. The oscillator triggering the protoplasmic streaming with a period of the 1 min range has been claimed to involve an internal Ca/cyclic AMP loop rather than periodic transport of ions through the plasma membrane (for review, see Stockem and Brix 1994). As shown here, the clock governing the contraction and shuttle streaming rhythm within one macroplasmodium can be forced to run at significantly different rates, according to local temperature effects within the physiological range of 15–29 °C, with the contraction frequencies differing up to the four-fold (see Fig. 7, at 90 min). This strongly argues against a master oscillator hypothesis. The temperature coefficient of local frequency roughly amounts to $Q_{10} = 2$, which indicates that the still unknown oscillator is based on chemical, rather than on electric, processes.

Achenbach and Wohlfarth-Bottermann (1980b) have analyzed thermally induced phase and frequency shifts of the shuttle streaming in isolated protoplasmic strands of 33 mm length which were exposed to a step-like temperature gradient. One third of the strand was kept at low temperature (17.7–21 °C), the middle third at medium temperature (22 °C), and the rest at high temperature (23.2–26.3 °C). The authors reported phase regulation of the shuttle streaming to occur up to a stepwise temperature gradient of 9 °C along a distance of 25 mm, which is half the value applied in our investigation.

In contrast to their experiments, our results have been obtained from freely moving macroplasmodia exposed to a smooth linear temperature gradient. Nevertheless our results fit well with the observations of Achenbach and Wohlfarth-Bottermann (1980b). The different frequencies of the contraction–relaxation cycles of large and small plasmodia located along the same isotherm indicate some kind of homeostasis. Possibly the plasmodia integrate temperature perception by thermoreceptors yet to be identified, relative

to the total area or mass. This argues again for a global distribution of the oscillator. As an effect of the temperature gradient, our multistrip kymographic analysis revealed an immediate response of the contraction–relaxation cycles, with a progressive frequency decrease in the colder domains, and an increase in the warmer domains. As documented in Fig. 7, there are significant changes of the contraction frequency even in those domains of the macroplasmodium which maintained their local temperature of 26 °C when the gradient was established. Both the resynchronization and desynchronization processes involved here indicate that the applied temperature gradient has a long range effect.

Interestingly, the final local contraction frequency within the domains of the macroplasmodia located close to the warm pole was significantly lower than that observed in the small macroplasmodia located within the same temperature range. This finding again indicates that macroplasmodia can sense temperature differences of 2 °C over a distance of 3 mm, and it suggests that the mass of the protoplasm collectively controls the capacity of the oscillating system as a whole. Accordingly, frequency shifts caused by differences in local temperature up to 2 °C were completely equalized within 1 h (Fig. 7). In steeper gradients, however, resynchronization was prevented. The resynchronization process of the contraction–relaxation cycle within the plasmodium suggests that the oscillation is driven by numerous omnipresent dispersed independent oscillators that can be entrained in a common global temperature domain, but can be uncoupled by a step of just 2 °C, or more.

In addition to the 1 min oscillator affecting actomyosin contraction cycles, a mitotic oscillator operates in the plasmodium with a period of about 10 h, which is leading to transient activation of the ubiquitous mitotic regulator, CDC2 kinase. On several occasions a correlation between nuclear division and a reduced frequency and/or amplitude of the contraction–relaxation cycles has been noted (Sauer 1982). These results, however, are not consistent. With the ECIS technique (see above) applied over a long period which included three synchronous mitoses, a tight correlation between the two oscillatory activities in *Physarum* could not be confirmed (Halvorsrud et al. 1995a). In this respect it is interesting to note that there is a transient growth arrest at intervals of 9.3 h, as revealed by time-lapse filming. This period is very similar to the rhythm of synchronous mitoses under these conditions. However, it was not possible to establish concordance between the two events for the same plasmodium, because nuclei must be immobilized to a large extent in order to determine mitosis by film analysis, a procedure which precludes the observation of several mitotic cycles in one plasmodium (Wolf and Sauer 1978, 1982; Wolf et al. 1979). However, this observation may indicate that the oscillator may not influence directly the mitotic cycle, whereas the brief growth arrest may suggest that the activation and inactivation of the CDC2 kinase at mitosis (Cho and Sauer 1994) is associated with a global shift in the activity state of an elusive growth regulator.

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