Growth patterns of the slime mold *Physarum* on a nonuniform substrate

Ragnhild Halvorsrud and Geri Wagner
Department of Physics, University of Oslo, Box 1048 Blindern N-0316 Oslo, Norway
(Received 4 June 1997; revised manuscript received 3 September 1997)

The Myxomycete *Physarum polycephalum* has been grown on nonuniform substrates, where the nutrients were confined in separated drops of agar medium. Spatial and temporal aspects of the resulting growth structures were studied by time-lapse video techniques and analyzed using image processing software. The growth process on a linear substrate of drops can be described in terms of a searching phase alternating with a feeding phase. On a linear array of drops, the *Physarum* advanced uniformly after an initial lag phase. On a two-dimensional drop substrate two different growth regimes could be distinguished: branched growth was observed on substrates with small drop diameters and compact growth, similar to growth on uniform substrates, was observed on substrates with larger drop diameters. The drop size is a crucial parameter that mediates characteristic plasmodial morphologies. A crossover from branched to compact growth was observed in some of the experiments. A spatial correlation function was used that could quantitatively distinguish between the different growth regimes. [S1063-651X(98)05501-9]

PACS number(s): 87.22.As, 05.40.+j

I. INTRODUCTION

The general interest in growth and formation of patterns in biological systems has increased rapidly in recent years [1–9]. One of many interesting patterns in nature is the network of veins formed by the slime mold *Physarum polycephalum*. In the plasmodium stage, *Physarum* forms a single giant cell that can measure several inches in diameter.

Since the establishment of axenic culturing techniques in 1961 [10], the Myxomycete *Physarum polycephalum* has become an ideal research organism for the study of processes of general biological significance [11]. Much attention has been devoted to the intrinsic biorhythms characterizing the cell cycle [12] and the ectoplasmic contractions driving the reversible protoplasmic streaming [13,14]. *Physarum* has also been a prime research organism in developmental biology [15,16] and genetics [17] since it can be cultured in the laboratory throughout its entire life cycle. A *Physarum* plasmodium has no permanent structure but retains a characteristic form throughout growth and migration. During migration the plasmodium is organized into a fan-shaped front followed by a network of interconnected veins. The veins decrease in diameter towards the front region, eventually merging into a homogeneous sheet of cell contents at the edge.

Here, we are concerned with a pattern simpler than the network of veins, namely, the geometrical shape of the developing plasmodium. It is well known that the plasmodium grows steadily in a humid environment with adequate nutrition, interrupted about every 10–14 h by synchronous nuclear division [11,18]. Under such conditions the plasmodium is completely sedentary and forms an approximately isotropic structure [19]. In contrast, non-nutrient substrates generate plasmodia with directed structures, capable of migrating a few centimeters per hour [20–22]. Migration can be directed by external stimuli such as chemical gradients (chemotaxis) [23] and temperature gradients (thermotaxis) [24–26].

The composition of the substrate supporting a growing plasmodium is crucial with respect to the structure that is formed. The general knowledge of growth and development in *Physarum* is basically limited to plasmodia grown under controlled conditions, where the substrate is uniform. In nature, however, the plasmodium is exposed to a nonuniform environment. It is thus interesting to study plasmodial growth and development on nonuniform substrates, where the dynamics of the growth patterns are better revealed. Recently, *Physarum* has been grown on substrates where the nutrition is confined in separated agar medium drops. The drops had a diameter of about 1 cm, and were deposited onto a hydrophobic surface [27]. These substrates mediate an active search for nutrients, and nutrient absorption can only take place within confined regions. In contrast, uniform substrates allow for continual absorption of nutrients both spatially and temporally. Patterns of growth ranging from sedentary, dense growth to nonuniform, diffusive growth were observed.

Two different phases can be distinguished during plasmodial advancement on the drop substrate. During the ''searching phase,'' the plasmodium migrates on the open space between the nutrient drops. The ''feeding phase'' begins when a fresh drop is contacted by the plasmodium. The plasmodium covers the drop completely and remains stationary until the next searching phase begins, possibly triggered by nutrient exhaustion near the drop surface.

In this work, we report studies of the growth and migration of *Physarum* on the borderline between the sedentary and the migratory regime. Using drops with small diameters, we found that the patterns of invaded drops form random ramified structures, in pronounced contrast to the approximately circular shape of a sedentary plasmodium growing on a uniform plate of agar medium. A photograph from the final stage of one of the experiments is shown in Fig. 1. The plasmodial structure branched as the plasmodium searched for and found nutrient drops. The length scale of the branches was determined by the drop diameters and was much larger than the scale characterizing the branched searching structure of a starving plasmodium. In some of the

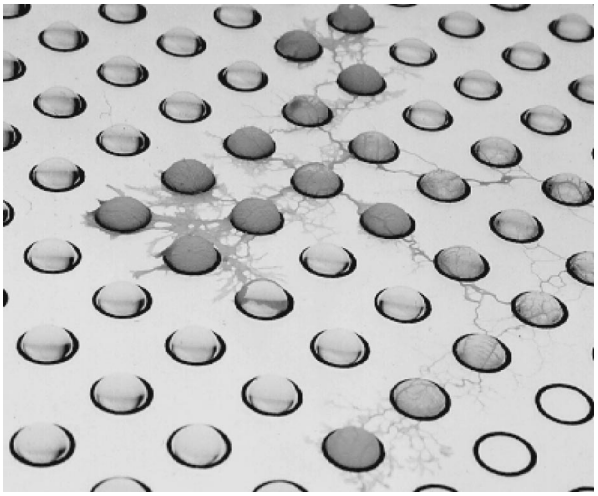

FIG. 1. *Physarum* growing on a substrate consisting of discrete agar medium drops. The most recently invaded drops are covered by yellowish plasmodium (shown in gray). Drops that became invaded at earlier stages are covered by a transparent structure. The substrate is of type *A*; see Table I.

experiments with intermediate drop diameters, a transition from random, ramified growth to compact growth—reminiscent of growth on a homogeneous substrate—was observed as the plasmodium size increased.

II. METHODS

In all of the experiments, the Colonia-Leicester strain (CL) [28] of *Physarum polycephalum* was used, which develops haploid plasmodia [29]. The CL strain is known to be less light sensitive than the diploid strains, tolerating weak light exposure without sporulating. The plasmodia were kept in an incubator at 27 °C and maintained in petri dishes on agar containing McArdles medium. Details of the culturing methods can be found elsewhere [11].

A. Preparing the substrate

Computer-drawn templates were used to prepare substrates with regular arrays of nutrient drops. The templates contained ink circles with the desired size and spacing. The pattern of circles was imprinted on hydrophobic transparency sheets. A mixture of molten agar and McArdles medium was deposited in the centers of the circles at a temperature of about 70 °C, using a multidispenser pipette (Finnpipette 4540). The resulting constant volume drops solidified in about a minute.

Linear arrays and two-dimensional square lattice arrays were prepared, including up to 30 and 22 × 22 drops, respectively. The lattice parameters used can be found in Table I. In the square lattice arrays, the drops covered between 23% and 67% of the total substrate area.

The substrates were inoculated by transferring a plasmodial sample to one of the drops. Samples were taken from vital plasmodia with a compact structure. In most cases, the inoculated substrate was kept in an incubator for some hours to optimize growth conditions. The first searching phase started after a long delay that varied greatly among the samples. The first invasion event was thus not included in the statistical analysis.

TABLE I. Parameters of the drop substrates used in the experiments, including the center-to-center distance δ between two neighboring drops, the drop diameter d, the drop volume V, and the fractional area covered by the drops.

Substrate	Spacing δ	Diameter d	Volume V	Coverage
A	0.92 cm	0.50 cm	25 μl	23%
B	0.92 cm	0.65 cm	50 μl	39%
C	0.92 cm	0.85 cm	75 μl	67%

B. Experimental setup

The inoculated substrate was contained in a transparent box made of 2 mm thick PMMA (polymethylmethacrylate) plates that was closed with Parafilm sealing tape. A diffuse light source was used to illuminate the transparent substrate. Four fans were mounted to disperse the heat emitted by the light source and to prevent condensation of vapor inside the experimental box.

The growth process was monitored using time-lapse video recording (Panasonic TL 6720A). The video camera (Panasonic WV-GL350) was interfaced with a PC through a PCVision Frame Grabber Adapter for on-line processing and analysis of the image. Based on the gray scale of the pixels representing the substrate, the system was able to distinguish between drops covered by plasmodial mass and bare drops (occupied and nonoccupied drops, respectively).

The duration of an experiment ranged from one day to a week, depending on the drop size and the drop spacing. All experiments were terminated when the plasmodial structure reached the boundary of the substrate.

C. Measurements and analysis

The experiments were analyzed in terms of invasion events in space and time. An invasion event was defined as propagation of the plasmodium onto a fresh nutrient drop. For each invasion event, the arrival time t and the drop position vector were measured.

The analysis of the growth process was done either manually by studying time-lapse video recordings, or automatically by means of an image processing routine. In the manual analysis, t was defined as the time when the *Physarum* structure physically contacted the new drop. In the automatic analysis, the experiment was recorded as a sequence of digitized black-and-white images with a resolution of 256×512 pixels. Each pixel represented a region of the substrate used in the experiment, and had a value corresponding to the brightness of the region. The arrival time was defined as the time when the majority of the pixels representing a drop had values below a threshold value, indicating covering by the plasmodium. The two methods were carefully compared and gave consistent results.

III. RESULTS

A. Growth on the drop substrate

Figure 2(a) shows a plasmodium growing on a one-dimensional drop substrate during the searching phase. The

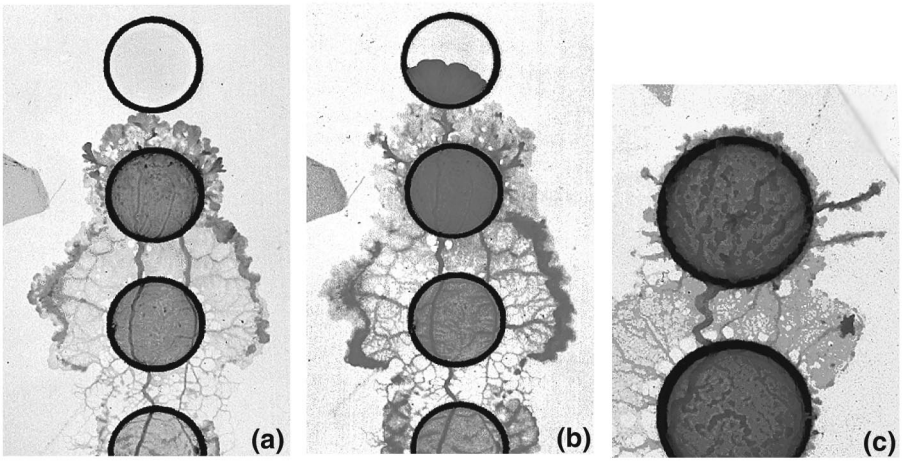

FIG. 2. *Physarum* growing on a regular one-dimensional array of agar medium drops of type *B* (medium-size drops). (a) The most recently invaded drop (second from top) is surrounded by a broad fan-shaped searching structure that is about to make contact with the next drop (searching phase). The searching structure formed after the previous invasion is also visible. The older, more transparent sections of the plasmodium were often organized into major veins oriented along the growth direction. (b) After 20 min, the plasmodium has entered the feeding phase, forming a smooth front as the drop is invaded. Expansion of the searching structures is stalled at this stage. (c) A finger-shaped searching structure protrudes from the plasmodium on a recently invaded drop, indicating the initiation of a new searching phase.

searching structures expanded almost isotropically within an angle of about 180°, with a slight preference for the forward direction. The expansion of a searching structure diminished strongly when the plasmodium made physical contact with a fresh drop, terminating the searching phase. In the feeding phase, Fig. 2(b), the plasmodium advanced rapidly on the fresh drop surface, forming a dense structure reminiscent of growth on a uniform substrate. Complete invasion of a drop was followed by a time delay before a new searching phase began, indicated by new plasmodial structures emerging outwards from the drop. Figure 2(c) shows a dendritic searching structure consisting of fingers protruding out from the plasmodium. The searching structures developed at any stage normally occurred in the direction along the major veins, much like the migration mechanisms observed when using homogeneous substrates [30,31]. In older parts of the plasmodium, the two phases could not be distinguished clearly, and were probably mixed. On two-dimensional substrates, different sections of the front changed from one phase to the other, such that the two phases coexisted.

During the growth process, the active part of the plasmodium resided on an increasing number of drops. Along with new invasions, protoplasm from aged structures was transported towards the front region. At a later stage, the aged structures could be reactivated if the plasmodium changed the direction of migration. The searching structures from previous invasion events eventually reorganized into thick veins, normally oriented along the propagation direction.

B. Growth on linear drop substrates

A drop at one end of a linear array of medium-sized drops (type B) was inoculated, and the plasmodium proceeded towards the other end. The number of invaded drops, $N(\tau)$, was measured as a function of the normalized arrival time, $\tau = t/T_N$, in 14 experiments, each involving 20 invasion events. For each experiment, T_N was defined as the mean time that elapsed between two successive invasion events. T_N varied from 44 to 108 min, reflecting large fluctuations among individual samples.

Figure 3 shows a plot of the number of invaded drops versus the averaged normalized arrival time $\overline{\tau}$, obtained by taking the mean of normalized arrival times with respect to a given number N of invaded drops. The data were fitted to a function of the form

$$N(\overline{\tau}) = -a + v\,\overline{\tau} + (1+a)e^{-\overline{\tau}/\tau_c}. \tag{1}$$

A nonlinear least-squares fit yielded $a = 4 \pm 1$, $v = 1.2 \pm 0.1$,

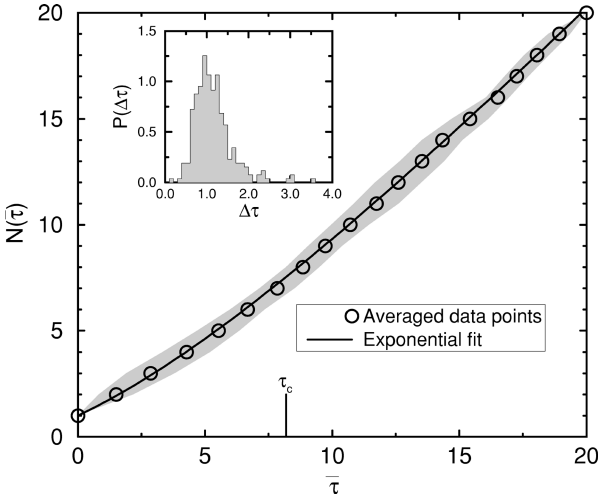

FIG. 3. The number $N(\overline{\tau})$ of invaded drops as a function of the averaged normalized time, $\overline{\tau}$, in 14 experiments on a one-dimensional array of drops (circles). The shaded region indicates the width of one standard deviation. The solid line represents a fit to the data points. Also indicated in the figure is the characteristic time τ_c, after which the growth became asymptotically linear in time. Inset: Probability density $P(\Delta\tau)$ of the normalized time interval $\Delta\tau$.

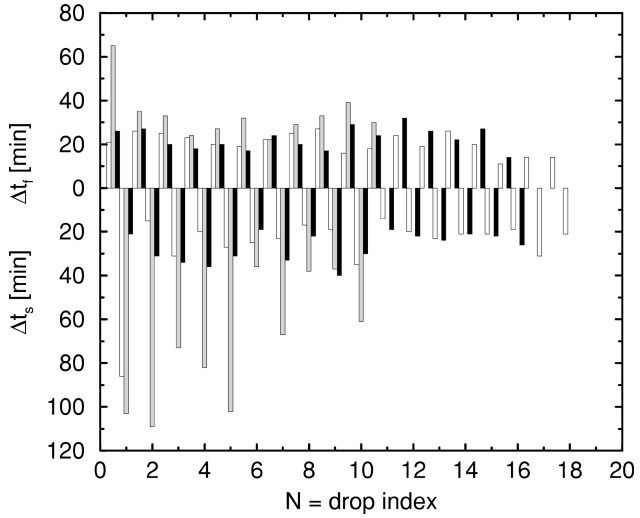

FIG. 4. Durations of the feeding phase and the searching phase, Δt_f and Δt_s, respectively, plotted as a function of the drop index N. Different gray shades refer to three different experiments conducted with linear drop substrates of type B.

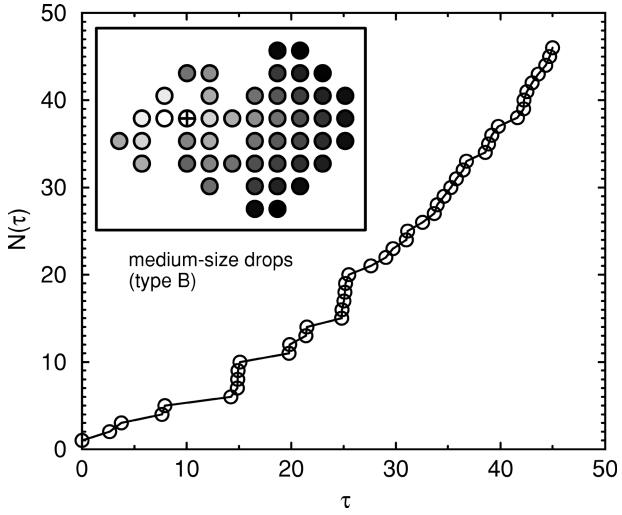

FIG. 5. The number $N(\tau)$ of drops invaded in an experiment using a two-dimensional substrate of type B, plotted as a function of the normalized time $\tau = t/T_N$. Inset: Graphical representation of the spatial growth pattern. The drops invaded by the plasmodium are represented by circles. The gray scale indicates the order of invasion, with the darker shades representing the later invasions. The inoculum drop is marked with a cross.

and $\tau_c = 8 \pm 2$. The data as well as the fit indicate a transient initial regime with reduced propagation velocity for $\overline{\tau} \ll \tau_c$, and a tendency of uniform advancement with constant propagation velocity, v, for $\overline{\tau} \gg \tau_c$. Thus a stationary state is approached after the time $\overline{\tau} \approx \tau_c$ (or $t \approx \tau_c T_N$) has elapsed.

The inset in Fig. 3 shows the probability density, $P(\Delta\tau)$, of the normalized time interval $\Delta\tau$ to elapse between successive invasion events. In most of the experiments, the invasions occurred very regularly, such that the intervals $\Delta\tau$ were clustered around 1.0. On some occasions, a plasmodium failed to hit the next drop within the average time range T_N, accounting for the tail towards long times.

Figure 4 shows the time intervals between successive invasion events, separated into intervals Δt_f and Δt_s. Here, $\Delta t_f(N)$ was defined as the elapsed time between the moment of first contact with the Nth drop and the complete coverage of the drop by the plasmodium. This interval was interpreted as the duration of the feeding phase. $\Delta t_s(N)$ represented the time from the complete coverage of the Nth drop to the moment of first contact with the next drop. This interval was interpreted as the duration of the searching phase. In the three analyzed experiments, Δt_s was systematically longer than Δt_f, and an average ratio of $\langle \Delta t_s / \Delta t_f \rangle \approx 1.5$ was found.

Using the values for the drop diameter and the drop spacing given in Table I, the migration velocities v_f and v_s during the feeding phase and the searching phase, respectively, was calculated. From the data shown in Fig. 4, a migration velocity ratio of $v_f / v_s \approx 3.5$ was found, indicating a reduced migration velocity in a fixed direction in non-nutrient spaces.

C. Growth on two-dimensional drop substrates

Three different substrate types (A, B, and C) were used (see Table I). The drop size was varied while the center-to-center drop distance was kept constant. A drop in the center of the substrate was inoculated, and the growth process was monitored until the substrate boundary was reached. Figure 5 shows the number of invaded drops, $N(\tau)$, as a function of the normalized arrival time, $\tau = t/T_N$, in a typical experiment using a type B substrate (medium-size drops). The substrate size was 13×13 drops, and the experiment lasted for 36 h. The inset shows the spatial pattern of invaded drops. The temporal order of the invasions is indicated by the increase in gray shades.

The growth patterns observed in the experiments were of great diversity. Figure 6 shows a graphical representation of three additional experiments on substrate B. In Fig. 6(a) the plasmodium grew in a branched manner and did not form a compact structure. In the experiments represented in Figs. 6(b) and 6(c), the substrates had the same properties (type B) but contained a 20×20 array of drops. In one of the experiments [Fig. 6(b)], the plasmodium grew in a compact manner and covered a large fraction of the drops.

In the experiment represented in Fig. 6(c), the plasmodium appeared to migrate in one direction rather than spreading out approximately isotropically.

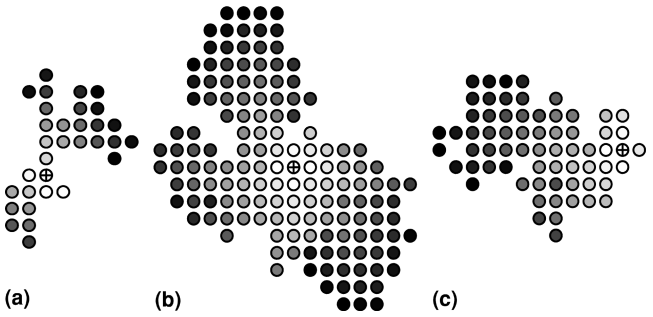

FIG. 6. Graphical representations of plasmodial growth in three experiments using two-dimensional substrates of type B (medium-size drops). The drops invaded by the plasmodium are represented by circles. The gray shade indicates the order of invasion, with the darker shades representing the later invasions. The inoculum drop is marked with a cross.

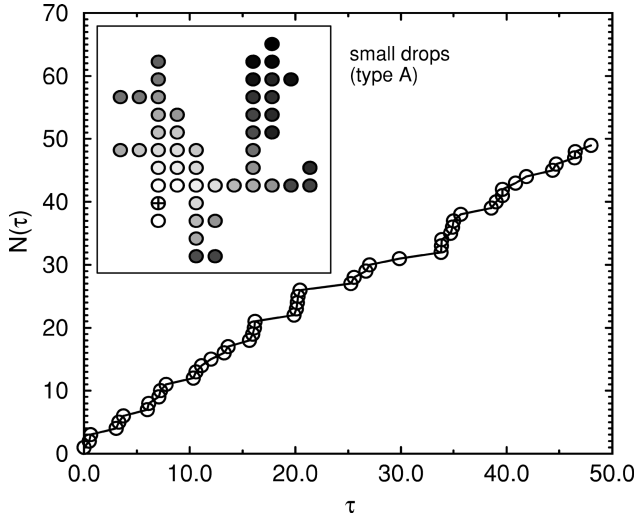

FIG. 7. The number $N(\tau)$ of drops invaded in an experiment using a two-dimensional substrate of type A (small drops), plotted as a function of the normalized time $\tau = t/T_N$. Inset: Graphical representation of the spatial growth pattern.

Figures 7 and 8 show graphs analogous to Fig. 5 obtained from two experiments in which smaller drops (substrate type A) and larger drops (substrate type C) were used, respectively. The substrates sizes were 21×21 drops in both cases. The plasmodium appeared to grow in a random manner in a low-nutrient environment (Fig. 7). Growth usually occurred in only one direction at a time and the plasmodium formed branches. At any stage, the growth direction could reverse and new branches were formed. The invasion of drops occurred in bursts, as the growing searching structure found several fresh drops approximately simultaneously. In a more nutritious environment (Fig. 8), bursts occurred only during the initial stage. Several searching structures originating from different drops developed simultaneously, and the burstlike invasion pattern was smeared out. At later stages, the plasmodium formed a more compact structure consisting

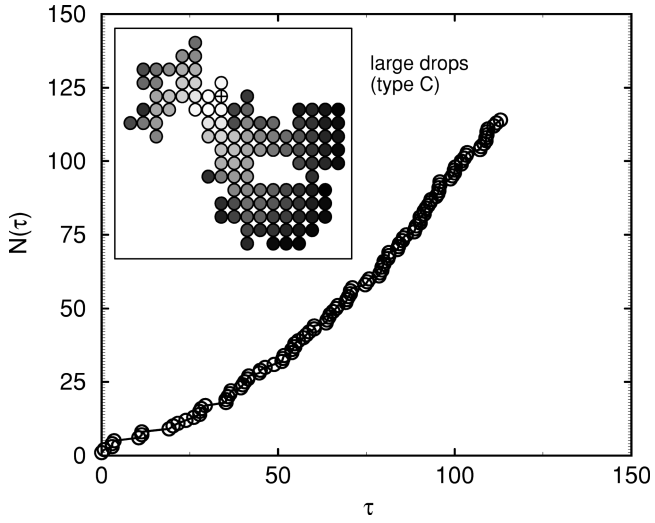

FIG. 8. The number $N(\tau)$ of drops invaded in an experiment using a two-dimensional substrate of type C (large drops), plotted as a function of the normalized time $\tau = t/T_N$. Inset: Graphical representation of the spatial growth pattern.

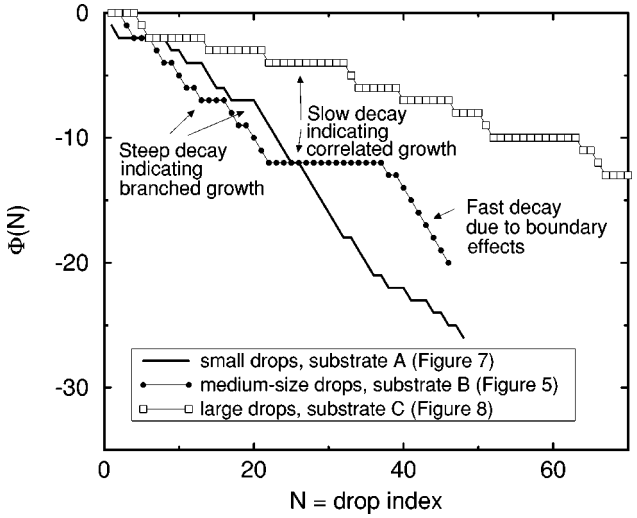

FIG. 9. Plot of the function $\phi(N)$ defined in Eq. (3) as a function of the drop index N. The curves correspond to the experiments illustrated in Figs. 5, 7, and 9.

of one or more massive regions of invaded drops. The plasmodium expanded continuously along a wide front, and the number of invaded drops increased approximately quadratically with time.

In the experiment illustrated in Fig. 5, the growth pattern underwent a transition from branched growth to compact growth after about 20 h ($\tau \approx 30$). The transition is reflected in a smooth increase of $N(\tau)$ for $\tau > 30$, compared to the stepwise increase in the early stages.

The difference between the two growth regimes can be quantified by defining a function $1 \leqslant \chi(N) \leqslant 4$ representing the number of occupied drops among the nearest neighbors of the Nth drop invaded by the plasmodium. $\chi(N)$ is a measure for the spatial correlation in the vicinity of the Nth drop, observed at the final stage of the experiment. A coarser description is more suitable, provided by the step function

$$\varphi(N) = \begin{cases} 1 & \text{if } \chi(N) \geqslant 3 \\ 0 & \text{otherwise.} \end{cases} \tag{2}$$

$\varphi(N)$ is defined for $0 \leqslant N \leqslant N_{\max}$, where $N_{\max}$ is the maximum number of drops invaded during an experiment. The threshold coordination number 3 was chosen judiciously. By integrating $\varphi(N)$, a continuously decreasing correlation function

$$\phi(N) = \left(\sum_{i=1}^{N} \varphi(i) \right) - N \tag{3}$$

is obtained, characterizing the temporal evolution of the spatial correlations during an experiment. Here, the drop index N is used as a time parameter. (The subtraction of N is only for convenience.) For branched growth of the plasmodium, most of the drops will have less than three invaded neighbors and $\phi(N)$ decreases with a slope close to -1. If a compact cluster of invaded drops is formed, the drops in the center of the cluster have three or even four invaded neighbors, and $\phi(N)$ is constant.

Figure 9 shows a plot of $\phi(N)$ as a function of N for the

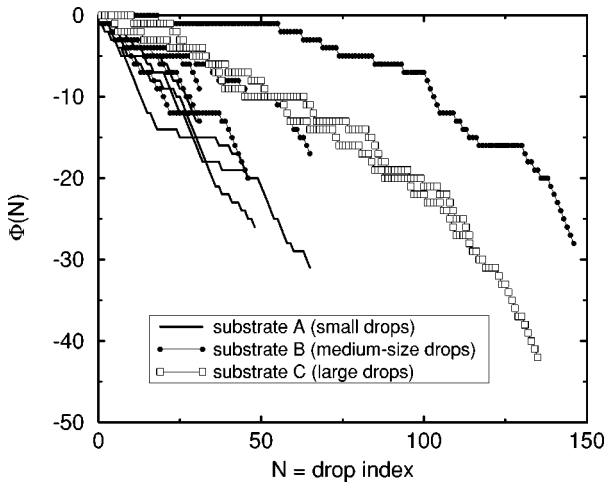

FIG. 10. Plot of the function $\phi(N)$ defined in Eq. (3) as a function of the drop index N. The curves correspond to experiments performed on substrates of type A, B, and C. The data from Fig. 9 are included in the plot.

three experiments illustrated in Figs. 5, 7, and 8. For the experiment on the type A substrate (Fig. 7), $\phi(N)$ decreased with a slope of about -0.5. For the experiment on the type B substrate (Fig. 5), $\phi(N)$ reached a plateau at $N \approx 20$, after an initial decay. The plateau indicates that drops invaded at this stage became part of a compact plasmodial structure. For the experiment on the type C substrate (Fig. 8), $\phi(N)$ had several small plateaus and decreased slowly.

Figure 10 shows a similar plot of $\phi(N)$ representing eleven experiments. Plateaus reflecting a crossover from branched to compact growth are apparent in the graphs representing experiments on type B substrates (medium-size drops). Using type A substrates (small drops), a similar crossover occurred in one experiment whereas three plasmodia grew in a branched fashion, indicated by the fast decrease of $\phi(N)$. In two experiments on type C substrates (large drops), the plasmodia formed compact structures at early stages such that $\phi(N)$ decreased slowly, in the absence of dominant plateaus. An even slower decrease of $\phi(N)$ was measured in one of the experiments using a type B substrate [represented in Fig. 6(b)] in which the plasmodium formed an extremely compact cluster.

IV. DISCUSSION

In the present work, an experimental approach was developed to study the patterns of growth in *Physarum* plasmodia from a macroscopic point of view, using nonuniform substrates. The plasmodial behavior on these substrates comprises a feeding phase characterized by growth (increase of the plasmodium mass by nutrient absorption from a drop), and a searching phase characterized by migration of the plasmodial front in a non-nutrient area (without any local increase of the plasmodial mass). The major veins formed between previously invaded drops provide an effective connection, allowing rapid transport of protoplasm all along the structure. In comparison, sedentary plasmodia on homogeneous substrates normally arrange the major veins concentrically. The growth was quantified using the spatial correlation function $\phi(N)$ defined in Eq. (3).

Plasmodial growth of *Physarum* growth has previously been characterized in terms of RNA, DNA, and protein content [32,33]. In suspensions of microplasmodia [19], a constant protein to DNA ratio has been observed during the exponential phase [34,35], indicating that nuclear division, macromolecular synthesis, and overall growth are closely related [12]. However, the biochemical processes in the cell have not yet been related to the growth and development of the plasmodium on the *macroscopic* scale. Significant progress in this field may be expected from the use of mathematical models in which the growth processes are reduced to a set of simple rules or differential equations. Recent examples include the description of bacterial growth [36,37] and aggregation of amoebae [38], stripe formation on the skin of fish [39], and pattern formation on sea shells [40]. The calibration of any such numerical model in terms of time scales and length scales is a difficult issue. In the present work, a time scale was defined by the sequence of drop invasion events during each experiment. The experiments described here are particularly well suited for comparison with a numerical model, because growth patterns of individual plasmodia and of simulated plasmodia could be compared in terms of drop invasions and alternating ''searching'' and ''feeding,'' rather than in terms of absolute time and length. A computer model for the plasmodial growth is now under development [48]. Preliminary results indicate that two conditions are crucial to match the experimental observations of growth on a drop substrate: (a) a strong preference to grow well-supplied structures in nutritious regions, and (b) a strong preference to expand plasmodial regions that were formed recently.

On one-dimensional drop substrates, the plasmodia followed the trace of nutritious regions and the asymptotic growth velocity was constant. On regular two-dimensional arrays the plasmodia formed irregular structures. The growth appeared to be a random process, and the shape of the evolving plasmodia varied greatly within a series of experiments. However, the plasmodia tended to grow in a more sparse manner on substrates with a low density of nutrient. On substrates with a higher nutrient concentration (larger drops), the plasmodia grew more compactly, but without attaining the almost regular shape of a sedentary plasmodium on a uniform plate of agar. In other words, the size of the drop is a crucial parameter that mediates characteristic plasmodial morphologies. This result has strong parallels with observations in other systems, as in bacterial colonies, where the overall pattern of growth can be externally controlled by varying the concentration of nutrients or the stiffness of the substrate [2,4].

In some of the experiments with substrates of intermediate drop size, a gradual transformation from branched growth to compact growth was observed. Possibly, the crossover would have occurred in all the experiments with intermediate drop sizes if sufficiently large substrates had been used. It might be speculated that such a crossover is related to the acquisition of a critical density of propagating fronts of the plasmodium. We have, however, not been able to connect the crossover with a unique order parameter. Relatively large variations were observed in the mean time, T_N, elapsing between two successive invasions (up to a factor of about 2.5 for different experiments). Furthermore, the initial delay be-

tween inoculation and the first invasion event was found to vary even more. These fluctuations would probably be greatly reduced by using coalescence of microplasmodia as an inoculation process. The plasmodium formed by coalescence is more isotropic compared to a piece excised from a large plasmodium. Improvement of the culturing methods may further reduce the fluctuations.

The growth of a plasmodium may be related to the cell cycle. Growth must alternate with the occurrence of mitosis such that a doubling of the mass between two successive mitoses occurs [19]. Consequently, growth must be related to the timing of mitosis, if balance is to be maintained. In our experiments, mitosis was observed after the invasion of approximately 5–10 drops on linear substrates [41]. The mitotic events were poorly synchronized, with a delay of up to 5 min between neighboring drops. This delay can be explained by the fact that an optimal mitotic synchrony relies upon sufficient cytoplasmic exchange between all parts of the plasmodium. Evidently, the drop substrates do not mediate structures with good cytoplasmic mixing.

It is commonly acknowledged that plasmodial migration ceases during mitosis [42–44]. Because of the asynchrony of mitosis between neighboring drops, propagation of the plasmodium can potentially be delayed. This may be a contributing factor to the reduction of propagation velocity on the linear drop arrays in the initial interval ($\overline{\tau} \ll \tau_c$) (Fig. 3).

Substituting up to 20% of the medium drops with pure water agar drops did not affect the growth process significantly. No deviations of the time differences Δt between subsequent invasion events could be discerned. This result reflects the relative importance of water uptake in addition to nutrient absorption in plasmodia grown on the drop substrate. However, the plasmodia developed the characteristics of a senescent structure earlier than the plasmodia grown on control arrays. The way the plasmodium interacts with its substrate is reflected also in the structure of the plasma membrane. Plasmalemma invaginations are a characteristic feature of *Physarum* in the plasmodium stage [45], resulting in an extensive enlargement of the surface area. It is reported that the number of plasmalemma invaginations is highly correlated with the nutritional content of the substrate [46]. Studies of the plasmalemma in connection with drop substrate might be revealing, since the substrate necessarily supplies discrete nutrient ''packages'' during the feeding phase.

In conclusion, contributions to a deeper understanding of the interplay between overall growth, nutrient absorption, and nuclear division rely on experiments in which the pattern formation on the macroscopic scale can be directly related to the cellular processes on a microscopic level. A suitable approach in this direction is to track the cell cycle continuously during the growth process without imposing any damage to the cell. This is possible for a plasmodium grown on a homogeneous substrate [47], but is more difficult on the drop substrate since the total cell mass is much smaller. Inhomogeneous substrates, on the other hand, may serve to magnify and quantify differences in cell morphologies that are controlled by external factors.

ACKNOWLEDGMENTS

We thank Morten Laane for performing experiments detecting mitosis, and Unni Oxaal, Jens Feder, Ivar Giaever, Paul Meakin, Torstein Jøssang, and Kate Clark for fruitful discussions and comments on the manuscript. We gratefully acknowledge the financial support from VISTA, a research cooperation between the Norwegian Academy of Science and Letters and Den norske stats oljeselskap a.s. (STATOIL) and by NFR, the Research Council of Norway.

[1] F. Family, B. R. Masters, and D. E. Platt, in *Fractals in Physics*, edited by A. Aharony and J. Feder (Elsevier, Amsterdam, 1989), pp. 98–103.
[2] M. Matsushita and H. Fujikawa, Physica A **168**, 498 (1990).
[3] Y.-K. Ng and P. M. Jannaccone, Developmental Biol. **151**, 419 (1992).
[4] E. Ben-Jacob *et al.*, Nature (London) **368**, 46 (1994).
[5] J. Pancio and P. Sterling, J. Comp. Neurol. **361**, 479 (1995).
[6] T. Höefer, J. A. Sherratt, and P. K. Maini, Physica D **85**, 425 (1995).
[7] A. D. M. Rayner, in *A Century of Mycology*, edited by B. C. Sutton (Cambridge University Press, Cambridge, 1996), pp. 193–232.
[8] J. O. Kessler, Math. Comp. Simul. **40**, 359 (1996).
[9] Y. Miyake *et al.*, J. Theor. Biol. **178**, 341 (1996).
[10] J. W. Daniel and H. P. Rusch, J. Gen. Microbiol. **25**, 47 (1961).
[11] J. M. Ashworth and J. Dee, *The Biology of Slime Moulds* (Edward Arnold Ltd., London, 1975).
[12] J. J. Tyson, in *Cell Biology of Physarum and Didymium*, Vol. I, edited by H. C. Aldrich and J. W. Daniel (Academic Press, New York, 1982), pp. 61–110.
[13] W. Stockem and K. Brix, Int. Rev. Cytol. **149**, 145 (1994).
[14] H. Komnick, W. Stockem, and K. E. Wohlfarth-Bottermann, Int. Rev. Cytol. **34**, 169 (1973).
[15] R. J. Wick and H. W. Sauer, in *Cell Biology of Physarum and Didymium*, Vol. 2, edited by H. C. Aldrich and J. W. Daniel (Academic Press, New York, 1982), pp. 1–20.
[16] J. Bailey, Microbiology **141**, 2355 (1995).
[17] J. Dee, in *Cell Biology of Physarum and Didymium*, Vol. 1, edited by H. C. Aldrich and J. W. Daniel (Academic Press, New York, 1982), pp. 211–251.
[18] E. Guttes and S. Guttes, in *Methods in Cell Physiology*, edited by D. M. Prescott (Academic Press, New York, 1964), pp. 43–54.
[19] H. W. Sauer, *Developmental Biology of Physarum* (Cambridge University Press, Cambridge, UK, 1982).
[20] N. Kamiya, Ann. Rep. Sci. Works Osaka Univ. **1**, 53 (1953).
[21] D. M. Miller, Exp. Physiol. Biochem. **4**, 183 (1971).
[22] J. Dee, Sci. Prog. Oxf. **62**, 523 (1975).
[23] T. Ueda and Y. Kobatake, in *Cell Biology of Physarum and Didymium* (Ref. [12]), pp. 111–143.
[24] K. Matsumoto, T. Ueda, and Y. Kobatake, J. Theor. Biol. **122**, 339 (1986).

[25] Z. Hejnowicz and K. E. Wohlfarth-Bottermann, Planta **150**, 144 (1980).
[26] W. Tso and T. E. Mansour, Behavioral Biol. **14**, 499 (1975).
[27] R. Halvorsrud, I. Giaever, and J. Feder, Biological Rhythm Res. **28**, 358 (1997).
[28] D. J. Cooke and J. Dee, Genet. Res. **23**, 307 (1974).
[29] M. M. Laane, F. B. Haugli, and T. R. Mellem, Norwegian J. Bot. **23**, 177 (1976).
[30] W. H. Lewis, in *The Structure of Protoplasm*, edited by W. Seifriz (Iowa State College Press, Ames, 1942), pp. 163–197.
[31] D. Kessler, in *Cell Biology of Physarum and Didymium* (Ref. [12]), pp. 145–196.
[32] J. W. Daniel and H. H. Baldwin, in *Methods in Cell Physiology*, edited by D. M. Prescott (Academic Press, New York, 1964), pp. 9–41.
[33] J. Mohberg and H. P. Rusch, J. Bacteriol. **97**, 1411 (1969).
[34] J. Mohberg, K. L. Babock, F. B. Haugli, and H. P. Rusch, Dev. Biol. **34**, 228 (1973).
[35] B. S. Plaut and G. Turnock, Mol. Gen. Genet. **137**, 211 (1975).
[36] E. Ben-Jacob *et al.*, Fractals **3**, 849 (1995).
[37] A. Czirok, E. Ben-Jacob, I. Cohen, and T. Vicsek, Phys. Rev. E **54**, 1791 (1996).
[38] D. A. Kessler and H. Levine, Phys. Rev. E **48**, 4801 (1993).
[39] S. Kondo and R. Asai, Nature (London) **376**, 765 (1995).
[40] H. Meinhardt, *The Algorithmic Beauty of Sea Shells* (Springer-Verlag, Berlin, 1995).
[41] M. M. Laane (private communication).
[42] K. Gotoh and K. Kuroda, Cell Motility **2**, 173 (1982).
[43] E. Guttes and S. Guttes, Exp. Cell Res. **30**, 242 (1963).
[44] C. Nations, A. Guevara, and I. L. Cameron, Exp. Cell Res. **132**, 493 (1981).
[45] R. P. Rhea, J. Ultrastruct. Res. **15**, 349 (1966).
[46] F. Achenbach, W. Naib-Majani, and K. E. Wohlfarth-Bottermann, J. Cell. Sci. **36**, 355 (1979).
[47] R. Halvorsrud, I. Giaever, and M. M. Laane, Protoplasma **188**, 12 (1995).
[48] G. Wagner, R. Halvorsrud, and P. Meakin (unpublished).