Oscillations and Morphogenesis

Edited by
Ludger Rensing
University of Bremen
Bremen, Germany

Marcel Dekker, Inc. New York • Basel • Hong Kong

Contents

Series Introduction *iii*
Preface *v*
Contributors *xi*

1 Introduction and Overview 1
Ludger Rensing and Andreas Deutsch

I Principles of Periodic Pattern Formation

2 Oscillations in Biological Pattern Formation 27
Hans Meinhardt

3 Controversy over Concepts: Long-Range Communication Versus "No Crosstalk" 43
Lionel G. Harrison and Thurston C. Lacalli

4 Spatial Patterns in Chemical Reactions 57
Stefan C. Müller

5 Stationary and Dynamic Patterns of Current Density in Gas Discharge Systems 81
Heinz Willebrand, Franz-Josef Niedernostheide, Renate Dohmen, and Hans-Georg Purwins

6 Oscillations and Morphogenesis: A Unifying Mathematical Approach 111
H. Haken

II Oscillatory Signals in Morphogenetic Fields

7 The Role of Periodic Signals in the Morphogenesis of *Dictyostelium discoideum* 133
Florian Siegert and Cornelis J. Weijer

8 Cells into Slugs: Oscillatory Temporal Signals, Taxis, and Morphogenesis in *Dictyostelium discoideum* 153
Michael G. Vicker

9 Intracellular Oscillations and Pattern Formation in the Cell Behavior of *Physarum* 167
Tetsuo Ueda

10 Growth Pulsations in Hydroid Polyps: Kinematics, Biological Role, and Cytophysiology 183
L. V. Beloussov, Ju. A. Labas, and N. I. Kazakova

11 Wavelike Propagation of cAMP and Ca^{2+} Signals: Link with Excitability and Oscillations 195
Albert Goldbeter and Geneviève Dupont

IIIA Periodic Timing of Morphogenetic Processes: Single or Repeated Transient Processes

12 Morphogenesis of *Acetabularia*: Coordinate Development, Spatial, and Circadian Control 213
Thérèse Vanden Driessche

13 Regulation of Cell Division Cycles by Circadian Oscillators: Role of the cAMP Pathway 227
Leland N. Edmunds, Jr.

14 Temporal and Spatial Gene Expression in Relation to Cell Division in the Pea Embryo 249
Angela J. Hauxwell, Fiona M. K. Corke, and Trevor L. Wang

15 Recurrent Activation Cycles, Not Localized Factors, Determine Cellular Bifurcations in Early Mosaic Development of Nematodes 259
Karl B. Moritz and Helmut W. Sauer

16 Oscillations, Photoperiodism, and Insect Development 277
David S. Saunders

IIIB Sequential Formation of Periodic Patterns

17 Concentric Rings in *Escherichia coli* Colonies 297
James A. Shapiro

18 Oscillators and Morphogenesis in Fungi 311
Stéphan Jerebzoff and Simmone Jerebzoff-Quintin

19 Morphogenesis of Periodic Conidiation Patterns in *Neurospora crassa*: Its Control by Circadian Rhythm and Daily Light and Temperature Signals 327
Ludger Rensing

20 Formation of Annual Rings in Trees 343
Rodney Arthur Savidge

21 Periodic Factors Influencing the Spatial Patterns in Bivalve Mollusk Shells 365
Christopher A. Richardson

IV Synchronous Formation of Periodic Patterns

22 Periodic Alignment of Chloroplasts in *Acetabularia* 381
Silvano Bonotto

23 Cell Movement and Pattern Formation in *Gonyaulax polyedra* 399
Till Roenneberg and J. Woodland Hastings

24 Spatial and Temporal Control of Ion Transport at the Plasma Membrane of *Chara corallina* 413
William J. Lucas

25 Dissipative Patterns of Ion Channels in Membranes 427
Peter Fromherz

26 Periodic Spatial Patterns in the Cellular Slime Mold *Polysphondylium pallidum* 437
Edward C. Cox

27 Pigment Cell Migration and Pattern Formation in Salamander Larvae 453
Lennart Olsson and Jan Löfberg

28 A Novel Cellular Automaton Approach to Pattern Formation by Filamentous Fungi 463
Andreas Deutsch

Index *481*

7
The Role of Periodic Signals in the Morphogenesis of *Dictyostelium discoideum*

Florian Siegert and Cornelis J. Weijer *Zoological Institute, University of Munich, Munich, Germany*

I. INTRODUCTION

Morphogenesis, the process by which cells differentiate and are organized in space and time, is a central problem in biology. There are two totally different possible mechanisms to set up a spatial pattern: (1) Cells differentiate in situ and the problem reduces to the generation of a spatial morphogen gradient. (2) Cells differentiate position independent and then move to their proper destination, a process called cell sorting. Morphogenesis in the cellular slime mold *Dictyostelium discoideum* is a prime example of the latter mechanism. Single cells collect in an aggregate by chemotaxis to periodic signals. In the aggregate the cells differentiate in a random fashion and then sort out to form a simple spatial pattern in the multicellular slug stage. The slug is a three-dimensional excitable medium and is organized by a variety of twisted scroll and helicoidal waves. The cellular slime mold therefore provides a suitable system for the study of the relationship between cellular communication, biological oscillations, and differentiation.

II. THE LIFE CYCLE

Amoebae of *Dictyostelium discoideum* live in the soil and feed on bacteria. They multiply as single cells. The developmental cycle is initiated when the food source is exhausted. Some cells on the substratum start to secrete periodic cyclic adenosine monophosphate (cAMP) signals. If surrounding cells detect the cAMP signal with their cell-surface cAMP re-

ceptors, they respond with the production of cAMP and chemotactic movement toward the autonomous oscillator (Durston, 1974a,b). This leads to periodic cell movement toward the aggregation center. Streams are formed by cell–cell contacts, and after 4 h all cells of a field are gathered in a multicellular aggregate (mound) consisting of 10^3 to 10^5 cells. The mound transforms either directly into the fruiting body, which consists of two cell types, the stalk cells and spores, or alternatively, into a migratory slug. External signals such as light, temperature, or humidity direct slug migration and regulate culmination.

In the mound stage, the cells begin to differentiate in at least three cell types—prestalk, prespore, and anterior-like cells—which are initially found distributed at random. They then sort out to form a relatively simple pattern with prestalk cells in the anterior one-quarter of the slug and prespore cells in the back three-quarters of the slug. The anterior-like cells are distributed at random in the prespore zone; they resemble prestalk cells but will sort out from them (Sternfeld and David, 1981). Morphogenesis from the tipped aggregate stage onward is guided by the tip, a prominent structure, which forms during the mound stage. The tip shows all the properties of a classical organizer (Spemann, 1938; Raper, 1940): It determines the polarity of the prespore–prestalk pattern and coordinates slug movement and fruiting body formation.

The cellular communication system during aggregation is already well characterized. Still largely unknown are the exact mechanisms by which cells in the later stages of the development communicate. There is, however, increasing evidence that periodic signals secreted by the tip and chemotaxis to these signals organize cell movement and differentiation in postaggregative development. In this chapter we summarize the evidence that all aspects of morphogenesis can be accounted for by a mechanism involving periodic signals and chemotactic cell sorting. We start with a short summary of what is known about these signals during early aggregation and then summarize the evidence for their involvement in slug morphogenesis.

III. EARLY AGGREGATION

The aggregation process is brought about by three cellular competences:

1. Periodic production and secretion of cAMP into the medium by the cells in the aggregation center (pacemaker).
2. Detection of this signal by surrounding cells via their cell-surface cAMP receptors, followed by amplification of the cAMP signal through the activation of adenylate cyclase (relay response). This

response shows adaptation, which ensures outward propagation of cyclic AMP waves. Extracellular cAMP is continuously destroyed by membrane-bound and secreted phosphodiesterases.

3. Chemotaxis toward increasing cAMP concentrations. Cells respond to temporal increases in cAMP and therefore cells aggregate unidirectionally in the direction of the aggregation center (Devreotes, 1989).

The pulsatile nature of the cAMP signal can be visualized using dark-field optics. Aggregating amoebae on nonnutrient agar show propagating waves of light and dark bands which form concentric rings and/or spirals (Fig. 1). The light bands are composed of elongated inwardly moving cells, while the amoebae in the dark bands are not moving directionally

Fig. 1 Aggregation waves seen in strain AX-2 on agar plates containing 2 m*M* caffeine. The cAMP waves are seen as optical density waves in a dark field. The waves move from the center outward. The waves' leading edge is seen as a small dark band where the cells are rounded up (cringe response) at the detection of cAMP. This band is followed by a lighter band of cells actively engaged in chemotaxis. A darker gray band follows where the cells sit around and stick out pseudopods in order to try to detect new signals. Several centers are visible, all are scroll waves.

(Alcantara and Monk, 1974). The relay of the cAMP signal is visible as an outward-moving ring or spiral wave.

Different strains and mutants show different dark-field patterns. In aggregation fields of the wildtype strain NC4, concentric rings are predominant (Durston, 1974a), while in the axenic strain AX-2 concentric rings are rare and inevitably convert to spirals during aggregation. Centers emitting concentric rings consist of cells that autonomously secrete periodic cAMP signals. Spiral centers are set up by local differences in cAMP concentration, which break the symmetry of cAMP diffusion and are self-preserving (i.e., they do not require autonomous signaling) (Durston, 1974b). Once initiated, the signal is relayed around a core of cells. The period of the signal and the wave propagation speed determine the diameter of the core. Spiral centers often oscillate with frequencies higher than those of concentric ring centers, because the core evolves to a minimum size that is defined by the minimal refractory period of the cells. This agrees with the continuous decrease in period length between successive dark-field spiral waves.

Another observation shows that early morphogenesis is influenced by the frequency of the oscillator. Caffeine, a specific inhibitor of the cAMP relay (Brenner and Thoms, 1984), reduces the wave propagation speed and oscillation frequency (Siegert and Weijer, 1989). At concentrations above 2 m*M* caffeine, one observes a widening of the circumference of the center loop during the aggregation process (Fig. 2). This process continues until the loop breaks up to form several small mounds at the end of aggregation, which shows that the cells are still able to oscillate autonomously.

During aggregation the frequency of cAMP waves increases, which results in a decrease of the outward-moving velocity of successive waves. This negative correlation between oscillation frequency and wave propagation speed, the so-called dispersion relation, is also observed in the Belousov–Zhabotinski reaction (Pagola et al., 1988; Dockery et al., 1988). The explanation for this phenomenon is that when frequency increases, successive waves travel through a not yet completely recovered medium, and therefore the medium takes a longer time to become excited, which results in a reduction of wave propagation speed. This implies that diffusion of the signal from cell to cell is not the rate-limiting step in wave propagation but is determined by the kinetics of the underlying chemical reaction.

To obtain stable spirals there has to be an influence of curvature of the spiral wavefront on wave propagation velocity (i.e., the wavefront close to the aggregation center), showing that high curvature should propagate more slowly than the wavefront farther away from the center (describing

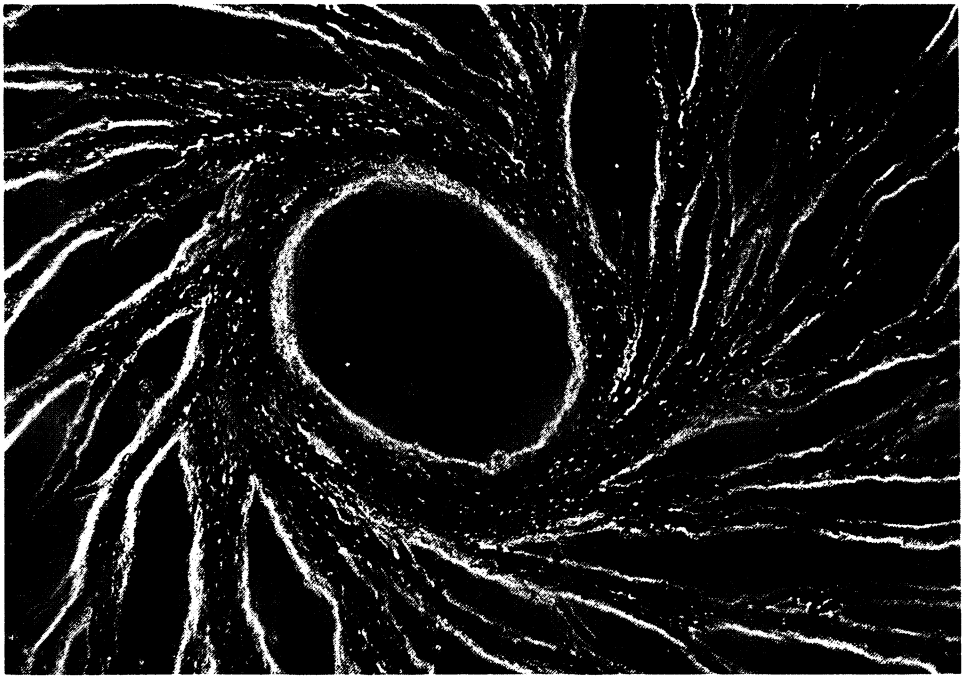


Fig. 2 Circular aggregation center of AX-2 cells aggregating on 2 m*M* caffeine. The cells rotate around in a loop while other cells are still flowing in from aggregation streams. Cell movement is clockwise and wave propagation is counterclockwise.

a greater radius of rotation). This curvature relationship was derived from theoretical model calculations (Keener and Tyson, 1986). For negative curvatures (waves curving away from the direction of propagation), the wave propagation velocity decreases, while for positive curvatures (waves curving in the direction of propagation as in the cusp of two colliding waves) it increases. This implies a minimum radius for the excitation center, below which wave propagation decays. Curvature was measured in spiral waves of both the Belousov–Zhabotinski reaction (Foerster et al., 1988) and in *Dictyostelium* and is in good agreement with theory. The minimum radius for aggregation centers on 2 m*M* caffeine was calculated to be 130 μm (Foerster et al., 1990). Our own calculations, based on signal propagation velocity and frequency, resulted in a radius of 100 to 200 μm.

IV. LATE AGGREGATION

Dark-field waves can only be seen for about 20 periods during early aggregation and disappear when cells form aggregation streams. The cAMP signaling system is difficult to study later in development since cell behavior is not visible, due to firm cell–cell contacts. A few reports based mainly on the movement of neutral red-stained cells have been published (Durston and Vork, 1979; Clark and Steck, 1979; Takeuchi et al., 1988). We have developed a method whereby we label cells fluorescently (with the cell type nonspecific label rhodamine–dextran) at the beginning of development and study the movement and behavior of individual cells in streams and slugs. Once loaded into the cells the rhodamine–dextran stays evenly dispersed in the cytoplasm until final differentiation. Digital image processing allows us to follow the tracks of several single cells simultaneously and to analyze velocity, frequency, direction of movement, and changes in cell shape (Siegert and Weijer, 1991).

Figure 3 shows that cell movement is periodic during early aggregation, in streams, and in slugs. Just before making cell–cell contacts, single cells of the axenic strain AX-2 move with an average period of 4.95 min, cells in streams with an average period of 3.3 min (Fig. 4). This confirms our earlier observation (Siegert and Weijer, 1989) that the frequency of cAMP signal propagation decreases during aggregation and decreases further after the disappearance of dark-field waves. As can be seen in Fig. 3, individual cells do move periodically in streams, although the periodicity is not as clear as in the dark-field waves. This must be attributed to the fact that we are now looking to the response of an individual cell and not to a population response as in the case of dark-field waves.

V. POSTAGGREGATIVE DEVELOPMENT

A. Evidence for Periodic Signals in Slugs

The main objective of this chapter is to summarize the evidence for periodic signaling and chemotaxis in the slug and culmination stages of development. During later development all morphogenetic movements are organized by the tip, a distinct morphological structure, which is present from late aggregation onward. The tip behaves as an organizer because it will induce a secondary axis by taking over part of the tissue behind it to form a new secondary slug when it is grafted into the side of another slug (Rubin and Robertson, 1975). Transplantation experiments have shown that the tip inhibits the formation of new tips (Durston, 1976). If a tip is transplanted into a slug containing a tip, its success of

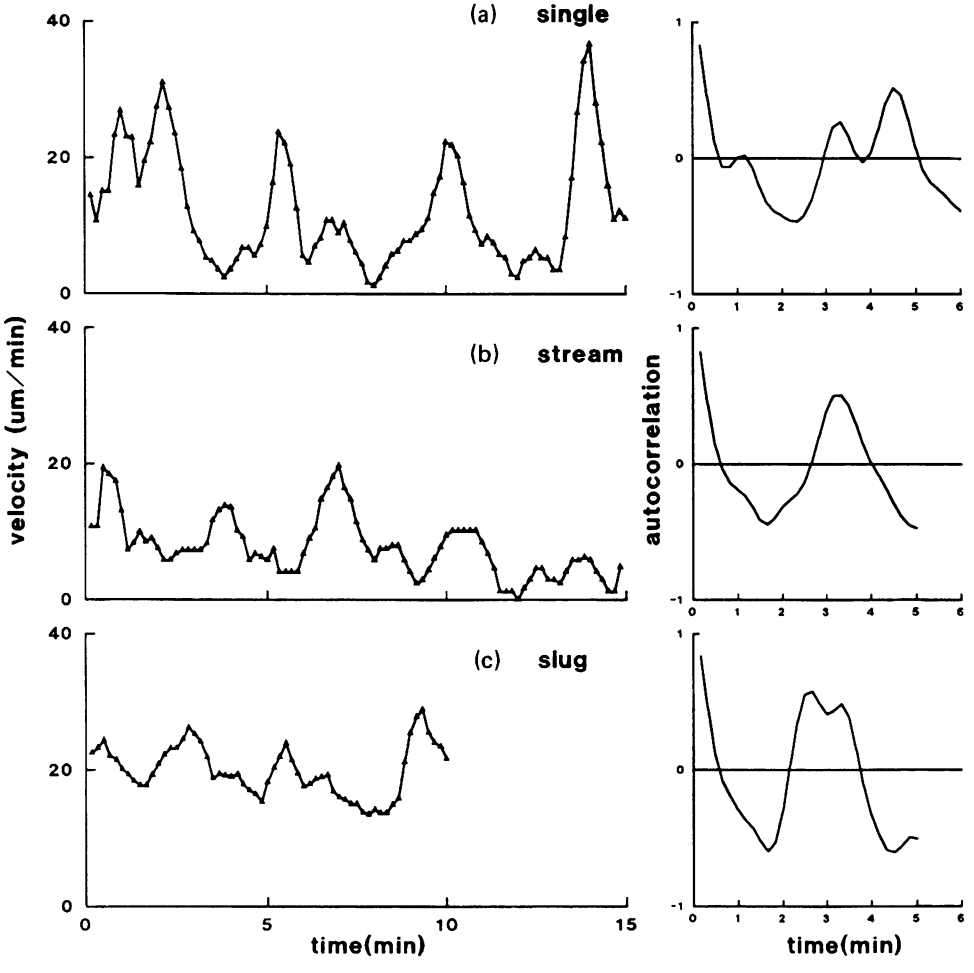


Fig. 3 Rate of movement of single cells at different developmental stages. The rate of movement of fluorescently labeled cells was measured by calculating the displacement of the cells' center of mass between two successive measurements (10 s). (a) Single cell in the early aggregation stage; (b) cell in an aggregation stream; (c) cell in the prespore zone of a slug. Also shown are the corresponding autocorrelation periodograms showing periods of movement between 3 and 2.5 min.

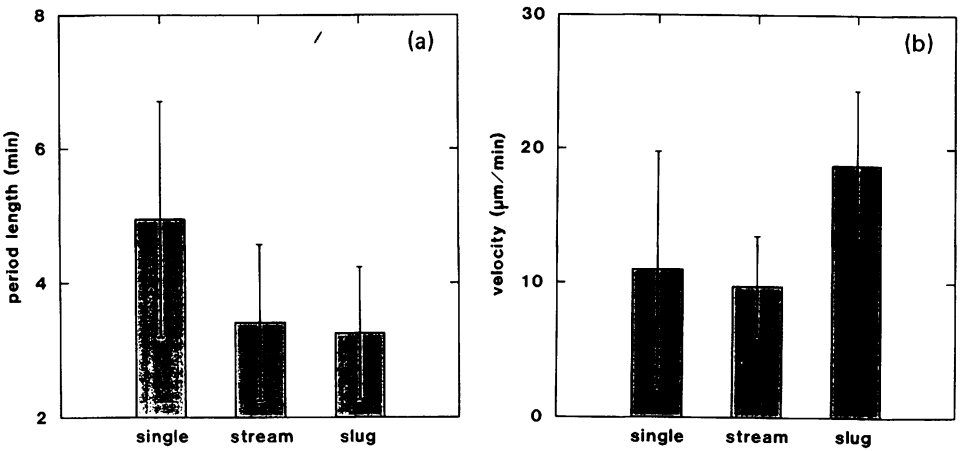


Fig. 4 Period and velocity of cell movement at different developmental stages: (a) mean period of cell movement for single cells at the stage before entering streams, in aggregation streams and in the prespore zones of slugs; (b) mean velocity of cell movement in single cells, cells in aggregation streams, and cells in the prespore zones of slugs.

forming a new tip is greater the larger the distance between the original tip and the grafted tip. If the slug is decapitated before tip transplantation, the rate of success of new tip formation increases dramatically. Tissue from the region of the tip is much more effective in inducing secondary tips in host slugs as tissue from the prespore zone of a slug, a phenomenon called tip activation (MacWilliams, 1982). Both these properties, tip activation and tip inhibition, can readily be explained by waves and oscillations. The tip inhibition signal is a periodic signal suppressing the emergence of autonomous centers, while tip activation is correlated with the excitability of the cells tested. The fact that prestalk cells form tips much more readily than prespore cells shows that prestalk cells are more excitable than prespore cells.

If the tip (less than 10% of the slug) is removed and placed on agar, it continues to move. The prespore piece stops immediately, rounds up, and forms a mound. The anterior-like cells in the prespore piece sort out and form a new tip. During this time some of the prespore cells redifferentiate to prestalk and anterior-like cells until a normal proportioned slug is formed and morphogenesis continues (Raper, 1940). This suggests that the tip coordinates cell differentiation and slug movement. These properties of the tip can be explained by assuming that it is a pacemaker for cAMP signals. Circumstantial evidence for periodic cAMP pulses as sig-

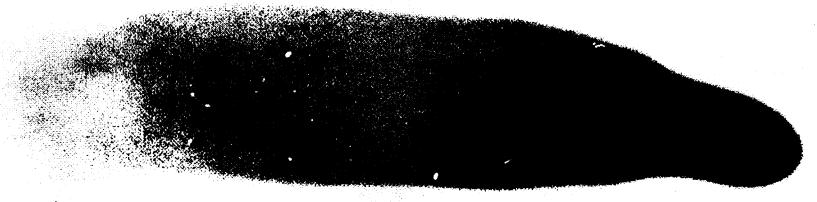
nal comes from experiments in which dissected slug tips have been shown to attract aggregation competent amoebae in a periodic fashion (Rubin and Robertson, 1975).

B. Relay Inhibitor Caffeine Removes Tip Inhibition

Further evidence for the involvement of periodic cAMP signals in pattern formation comes from experiments where slugs are treated with cAMP relay inhibitors. Neutral red-stained slugs placed on agar containing 5 m*M* caffeine stop migrating immediately. The anterior-like cells start to form local aggregation centers, precursor structures for new tips. The formation of these structures is completed after 2 h; however, further development into tips is blocked. After removal of caffeine these foci transform almost immediately into tips, which organize the surrounding tissue to slugs (Fig. 5). These experiments indicate that cAMP relay is the tip inhibition signal and that reduction of the signal amplitude and oscillation frequency by caffeine removes tip inhibition. The reduced oscillation frequency might be responsible for the inability of the aggregated anterior-like cells to complete tip formation.

C. Cell Sorting in Slugs Involves Chemotaxis to cAMP

There is good evidence that chemotaxis to cAMP is involved in cell sorting in slugs. If slugs, stained with the prestalk and anterior-like cell specific vital dye neutral red, are mixed on agar plates, the prestalk cells will sort out to the top of the aggregate, form a new tip, and then the aggregate forms a slug. If the same experiment is performed on plates containing cAMP, the prestalk cells will sort to the periphery of the aggregate, showing that prestalk cells sort preferentially to cAMP. It has also been shown that movement of the cells is directed, suggesting that the mechanism of movement is chemotaxis to cAMP (Matsukama and Durston, 1979). If dissociated neutral red-stained slug cells are mixed and reaggregated in suspension, the prestalk cells will sort to the center of the aggregate. If cAMP at 10^{-6} *M* is added to the buffer, the prestalk cells will sort to the periphery of the aggregate, showing that cAMP at this concentration interferes with the aggregate's own signaling system (Sternfeld and David, 1981).

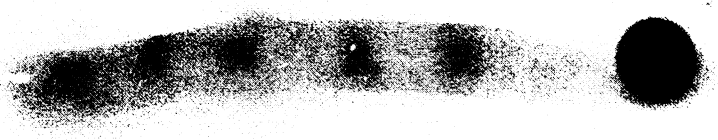


(a)

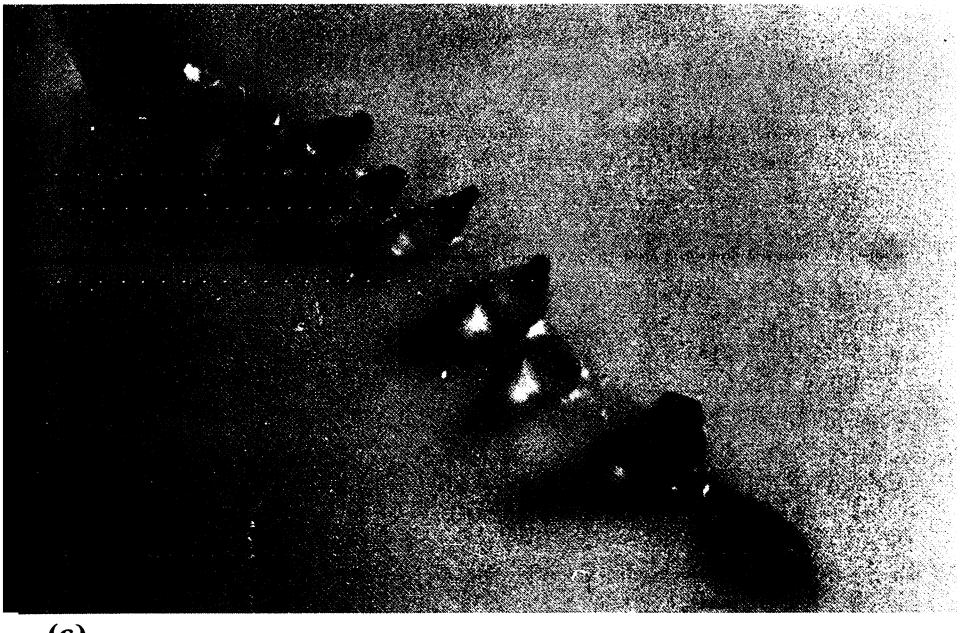
Fig. 5 Disappearance of tip inhibition in slugs on the relay inhibitor caffeine. (a) Photograph of a neutral red-stained slug. (b) Photograph of a slug after incubation on agar containing 5 m*M* caffeine for 2 h. The anterior-like cells in the prestalk zone have sorted out to form aggregation centers in the prespore zone of the slug. Development of the centers is arrested at a stage before tip formation. (c) Slug 2 h after being removed from caffeine containing agar to normal agar. The cells in the aggregation centers in the prespore zone have developed into tips characterized by small circumference. Each center normally forms a tip, except the original prestalk cells. Most likely they have irreversibly differentiated to stalk cells.

D. Cell Movement in Slugs Is Directed and Periodic

To test the hypothesis that the tip is a pacemaker and secretes periodic cAMP signals, it will be necessary to measure these signals in slugs. Until now it has not been possible to measure cAMP directly in three-dimensional structures as done for two-dimensional aggregation fields (Tomchik and Devreotes, 1981). Since the well-characterized dark-field waves are no longer visible in slugs, we used an indirect approach and investigated the cell movement response in slugs. By digital image processing we measured the velocity and periodicity of individual cells and the tracks



(b)



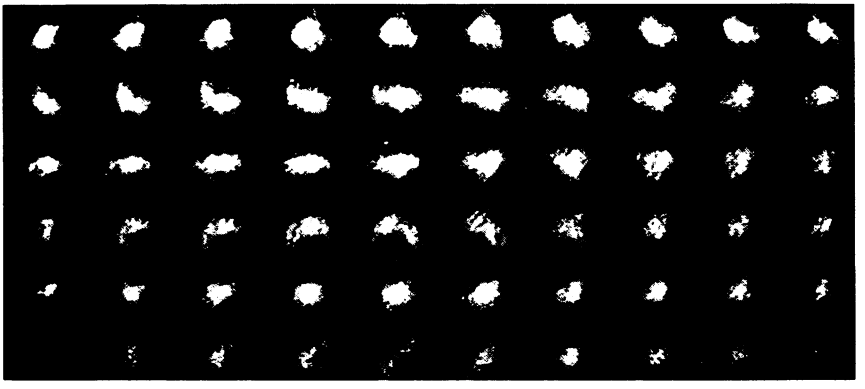
(c)

of many cells in a given slug. From these parameters we deduce signal periodicity and direction of wave propagation.

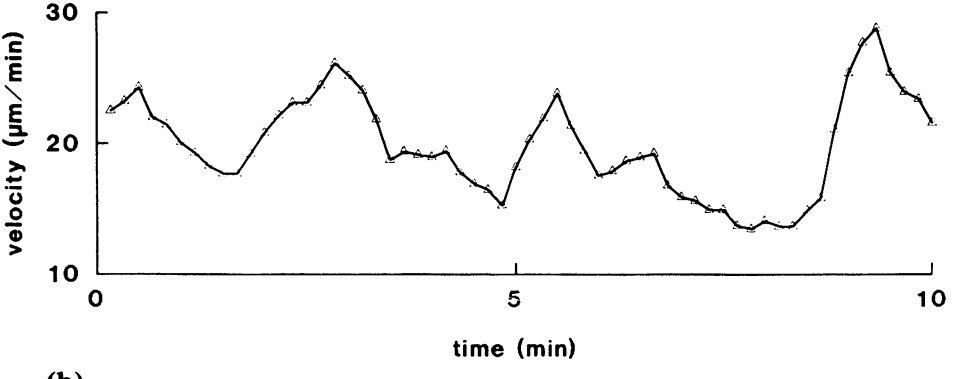
It appears that the movement of single cells in slugs is at least as periodic as that of cells in aggregation streams. Cells in slugs move with an average period of 3 min (Fig. 4). The periodicity of the signal is not as clear as in dark-field waves, for the same reasons as mentioned for aggregation streams (Fig. 3). Furthermore, it can be seen that the cells change their shape in a characteristic fashion during the periodic movement (Fig. 6). At the times of slow movement they stick out pseudopods in different directions before they decide in which direction to continue. This behavior is well known from cells moving chemotactically (Gerisch et al., 1975; Varnum-Finney et al., 1987) and shows that cells in slugs move in a chemotactic fashion. This technique allows us to investigate the precise movement of a few cells in a slug. To investigate the population behavior we developed a technique to follow many labeled cells in a slug (Siegert and Weijer, 1991). We found that cells in the prestalk zone of slugs move in a direction almost perpendicular to the long axis of the slugs, while cells in the prespore zone move straightforward (i.e., parallel to the long axis of the slug). Chemotactic movement perpendicular to the propagating wavefront in the prestalk zone implies that the wavefront must extend along the long axis of the tip. Therefore, the signal in the slug tip is a (twisted) scroll wave which decomposes in a wave traveling perpendicular to the long axis of the slug in the prespore zone (Fig. 7). An average slug length of 1 mm, a period length of 3 min, and a cAMP wave propagation speed of 200 μm/min (the lowest dark-field wave propagation velocity measured) imply that only one or two cAMP waves travel through a slug at a given point in time.

E. Model for Wave Propagation in Slugs

The decomposition of scroll waves into twisted scroll waves and then into planar waves was recently also observed in a three-dimensional Belousov–Zhabotinski reaction system along a concentration gradient of substrate (Yamaguchi and Müller, 1991). The change in the geometry of the propagating waves in *Dictyostelium* is in our opinion probably caused by a difference in the oscillatory properties of the prestalk and prespore cells. We have shown previously that one can separate aggregation stage cells that will sort to the tip of the slug from those that will sort to the back of the slug. The cells that will sort to the front have higher intrinsic oscillation frequencies than cells that will sort to the back of a slug (Weijer et al., 1984). Thus prestalk cells in the tip could dominate the remaining cells in the slug by their higher oscillation frequency. This



(a)



(b)

Fig. 6 Movement of a single cell in the prespore zone of a slug. (a) Composite picture showing the appearance of a fluorescently labeled cell in the prespore zone of a slug at 60 successive 10-s intervals. The series starts in the upper left corner and continues line by line from left to right to the last picture in the lower right corner. (b) Rate of movement of the cell shown in (a). The rate of movement is calculated as the displacement of the cells' center of mass over 10 s between two successive measurements. Every data point corresponds to a frame in (a).

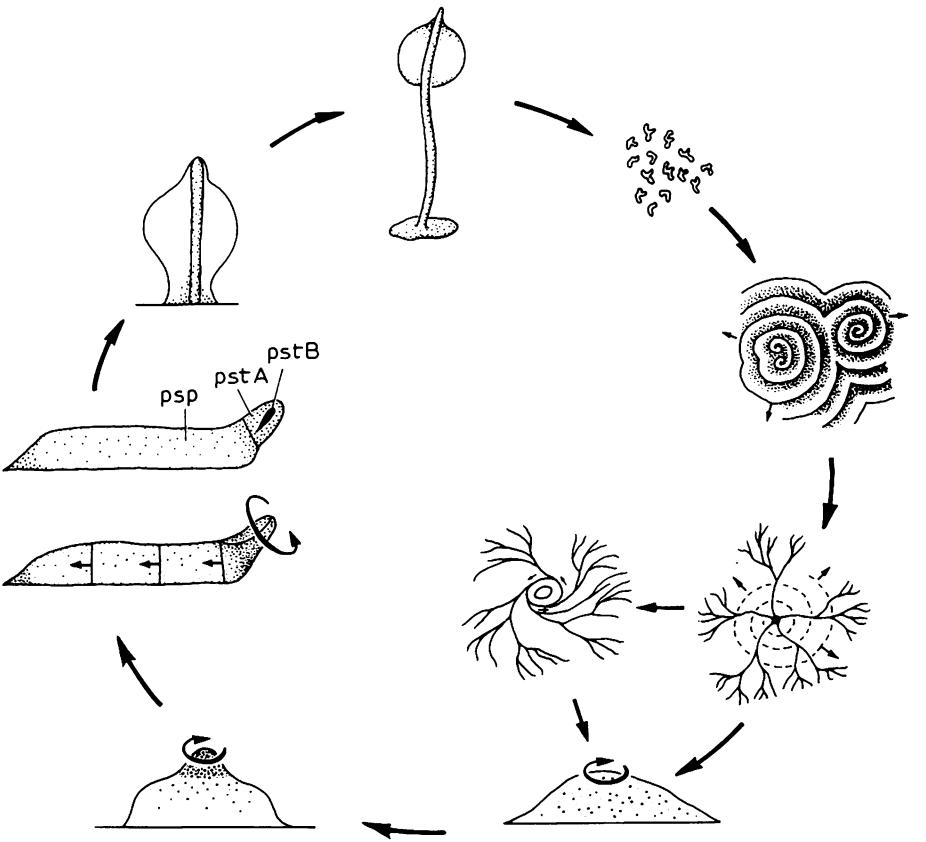


Fig. 7 Scheme showing the life cycle of *Dictyostelium discoideum* being organized by propagating waves and chemotaxis. The scheme starts with single cells that aggregate in spirals as seen in dark-field optics. After stream formation waves emanating from the center propagate through the aggregation streams, while the cells move inward. The aggregation center can either be a mass of cells or temporarily form a loop. After the cells have collected (mound stage), cell differentiation and cell sorting takes place. The prestalk cells sort out to form the tip. The tip is a twisted scroll wave. The wave rotates along its long axis as indicated by the black arrow. The mound now extends up in the air and forms a slug, which falls over and migrates away. The prestalk region stays a twisted scroll wave which decomposes on arrival in the prespore zone in planar waves, due to the lower excitability of the prespore cells. The core of the slug is a region of low cAMP, and this favors PST B expression, while the periphery of the tip with high cAMP favors PST A expression. The slug then converts by a series of not yet understood morphogenetic changes into a fruiting body, during which process the differentiation in the final cell types spore and stalk cells takes place.

would explain tip inhibition and formation of new slugs in grafting experiments. Furthermore, cells in the prespore zone can break down the external cAMP less efficiently than can cells in the prestalk zone, since they have less phosphodiesterase (Otte et al., 1986). This would leave them always being partly adapted. As a consequence, their autonomous oscillation frequency will be lower and the amplitude of the released signal will be smaller than that from the prestalk cells. A spiral wave collects all the cells into a mound during aggregation. There all the fast-responding cells (prestalk cells) sort out to the top of the mound, which leads to the formation of a three-dimensional twisted spiral organizer. The prestalk cells move toward the central core of the spiral. Since the spiral is twisted, this results in the formation and elongation of the tip. The circumference of the inner filament is determined by the minimal refractory period of the cells, with the diameter of the tip determined by the average refractory period of the cells. The twisted scroll wave then decomposes into planar waves in the less excitable prespore region, analogous to that observed in the Belousov–Zhabotinski reaction.

F. Models of Slug Movement and Behavior

One central question in slug morphogenesis is the mechanism of slug movement (i.e., how the motile effort of all cells in the slug is coordinated to result in migration). How is the fine tuning of cell movement achieved that results in the characteristic movement and shape changes? Several models of slug migration have been proposed. An intellectually attractive model is the "inverse fountain flow model" (Odell and Bonner, 1986). In this model cells in the periphery of the slug move forward until they reach the tip. From there they migrate again backward through the central core of the slug. Rotation of all cells in a slug is ruled out by the existence of a stable prespore/prestalk pattern, which would require a continuous redifferentiation of the cell types, which is not observed in the time scale of slug migration. If one assumes that this type of movement is restricted to either the tip or prespore zone, the prestalk zone has to be excluded since it is up in the air most of the time and one can not imagine how it can contribute to slug migration. If cell circulation takes place in the prespore zone, one would expect the cells in the core to be stationary in respect to the substratum and the cells in the periphery to move at twice the speed of the slug. This is not compatible with our observations since in our measurement all cells move with approximately slug speed.

In another model, the "squeeze-pull" model, it is proposed that cells gain traction from the slime sheath and the slug is embedded in an epithelial layer of specialized cells that can locally contract in a radial direction

that squeezes the anterior forward and can draw up the posterior part of the slug (Williams et al., 1986). This model would not explain the differences in movement behavior in the prestalk and prespore zone, and periodic cell movement would not necessarily follow. Furthermore, we found that slugs migrating under a coverslip in mineral oil can move without changing their diameter.

We propose that cells move chemotactically to periodic signals coming from the tip. The mechanism of coherent cell movement must be very similar to that in multicellular aggregation streams. The prestalk cells in the tip rotate in a twisted spiral and probably do not contribute motive force to slug migration. They are lifted above the substratum most of the time and serve as pacemaker. Motive force is produced mainly by the prespore cells, possibly through traction on an extracellular matrix between the cells which forms a continuum with the slime sheath surrounding the slug and which is left behind as a trail. Prestalk cells can produce a considerable motive force when placed in an agar tunnel (Inouye and Takeuchi, 1980). Their motive force per unit volume is even greater than that of a whole slug, which means that they move more vigorously than prespore cells. This is the prerequisite for the maintenance of a stable prestalk/prespore sorting pattern during development.

The active turning behavior in photo- and thermotaxis (Fischer et al., 1984) can be explained by differential cell movement due to slight modulation of the chemotactic signal or alternatively by a modulation of the intensity of the chemotactic response. Local changes in the relay properties of the cells will lead to distortion of the wavefronts and will be followed by changes in the direction of chemotactic movement. For example, the periodic up and down lifting of the slug tip (Williams et al., 1986) can be explained by differences in the rate of cell movement. Cells at the bottom move faster than upper cells due to loss of cAMP by diffusion in the substratum. As a result, the tip would be lifted up in the air until it falls back to the substratum and the process repeats itself again.

One factor that has been shown to influence morphogenesis drastically, most likely by modulating cell movement, is ammonia, a gas produced by starving cells. Ammonia orients the direction of fruiting body formation. Fruiting bodies will bend away from ammonia sources and move toward ammonia sinks. It probably acts by locally increasing the rate of cell movement (Bonner et al., 1986). Ammonia has been reported to stimulate both the rate of movement of vegetative amoebae and cells in aggregation streams at low concentrations and inhibit cell movement at high concentrations (Bonner et al., 1989). We have found that ammonia can increase the amplitude of optical density waves in early aggregation,

which we interpreted as a more intense response of cells to the chemotactic signal (Siegert and Weijer, 1989).

G. Role of Periodic Signals in Differentiation

It is well known that cAMP is not only involved in cellular communication but also in gene expression of both aggregation and slug stage-specific genes. The aggregation-specific genes, many of which are involved in the oscillatory cAMP pathway (i.e., cAMP receptors, cAMP phosphodiesterase, adenylate cyclase, and G proteins) are efficiently induced by periodic cAMP signals, while most of them are repressed by continuous flux of cAMP (Gerisch, 1987). Prespore specific genes are efficiently induced by both periodic and continuous signals (Schaap et al., 1986). Prestalk cell gene expression requires exposure to cAMP during the aggregation stage of development, followed by exposure to the morphogen DIF (Williams, 1988). Recently, it has been found that the prestalk zone of the slug consist of at least two classes of prestalk cells, PST A and PST B cells (Williams et al., 1989; Jermyn et al., 1989). Expression of the PST A cell markers is restricted to the front 10% of the slug; PST B cell markers are expressed in a central funnel that occupies 10 to 20% of the length of the slug (Fig. 7). Recently, it was reported that a specific combination of the morphogens DIF-1 and cAMP is necessary for the expression of the different cell markers (Berks and Kay, 1990): PST A differentiation is stimulated by cAMP and DIF-1, PST B differentiation is stimulated by DIF-1 and inhibited by cAMP, and prespore differentiation is stimulated by cAMP and inhibited by DIF-1.

With our concept of signal propagation in slugs we can offer an explanation for this peculiar pattern of cell differentiation. Cells in the center of the twisted scroll wave, which travels through the slug's tip, are adapted most of the time and therefore produce very little cAMP. Cells surrounding the central filament should give a response to every cAMP wavefront. This would lead to a low cAMP concentration in the central funnel and a higher cAMP concentration in the periphery. The low cAMP concentration in the central funnel will favor the expression of PST B, while the higher cAMP in the periphery favors expression of PST A.

VI. CONCLUSIONS

It is clear that there has to be a supercellular control mechanism to coordinate the behavior of a mass of single cells in the slime mold. *Dictyostelium* is first a two-dimensional and then later in development a three-dimensional excitable medium. On the basis of the data described above,

we believe that all of *Dictyostelium* development is controlled by periodic signals, wave propagation, and chemotaxis. Due to the developmental regulated increase in oscillation frequency and the corresponding decrease in wave propagation velocity, the system is able to organize both large aggregation fields and the much smaller slugs. The precise course of morphogenesis must be based on slight local changes in signal propagation and chemotactic responses.

Do Periodic Signals Controls Morphogenesis in Other Systems?

There are many examples of morphogenesis in the nonliving world that take place in excitable media. Well known are target patterns and spiral wave formation in the Belousov–Zhabotinski reaction, and the formation of hurricanes and spiral galaxies (Schulman and Seiden, 1986). One would, however, assume that morphogenesis in the living world involves excitable systems (Gierer and Meinhardt, 1972). Although it is clear that many biological patterns are composed of spatially periodic structures, it is less clear whether these are organized by temporarily periodic signals. Many biological processes show a temporal periodicity at all levels of organization. At the level of populations there are cycles of infectious diseases (Murray et al., 1986), reproduction, and extinction. Circadian rhythms occur in both population behavior and in individual's behavior and physiology. Individuals can show reproductive cycles, which are most often based on the reproduction of cells, which in itself again involves—among others—periodic DNA synthesis. Fewer examples are known of periodic information transmission as hormonal regulation and information processing in the nervous system. Only very limited data are available about the role of periodic signals in morphogenesis. The best documented example is certainly the cellular slime mold *Dictyostelium discoideum*. However, there is increasing evidence that the formation of periodic structures such as somites in vertebrates is controlled by a wave-like signal. This signal synchronizes the cell cycles of all the cells that will become allocated to a particular somite (Primmett et al., 1989). To what extent periodic cell movement and chemotaxis play a role in the morphogenesis of other organisms is still open to discussion. In some vertebrates, including the zebrafish and the mouse, there is extensive cell mixing before and after gastrulation. The significance and mechanism of cell movement in these animals is not yet well studied. It will therefore remain open to future experimentation to test the role of periodic signals and chemotactic cell movement in morphogenesis.

ACKNOWLEDGMENT

We thank the DFG (WE 1127) for support.

REFERENCES

Alcantara, F., and Monk, M. (1974). *J. Gen. Microbiol.*, *85*: 321.
Berks, M., and Kay, R. R. (1990). *Development*, *110*: 977.
Bonner, J. T., Suthers, H. B., and Odell, G. M. (1986). *Nature*, *323*: 630.
Bonner, J. T., Har, D., and Suthers, H. B. (1989). *Proc. Natl. Acad. USA*, *86*: 2733.
Brenner, M., and Thoms, S. (1984). *Dev. Biol.*, *101*: 136.
Clark, R. L., and Steck, T. L. (1979). *Science*, *204*: 1163.
Devreotes, P. N. (1989). *Science*, *245*: 1054.
Dockery, J. D., Keener, J. P., and Tyson, J. J. (1988). *Physica D*, *30*: 177.
Durston, A. J. (1974a). *Dev. Biol.*, *38*: 308.
Durston, A. J. (1974b). *Dev. Biol.*, *37*: 225.
Durston, A. J. (1976). *Nature*, *263*: 126.
Durston, A. J., and Vork, F. (1979). *J. Cell Sci.*, *36*: 261.
Fisher, P. R., Dohrmann, U., and Williams, K. L. (1984). *Mod. Cell Biol.*, *3*: 197.
Foerster, P., Müller, S. C., and Hess, B. (1988). *Science*, *241*: 685.
Foerster, P., Müller, S. C., and Hess, B. (1990). *Development*, *109*: 11.
Gerisch, G. (1987). *Annu. Rev. Biochem.*, *56*: 853.
Gerisch, G., Hülser, D., Malchow, D., and Wick, U. (1975). *Philos. Trans. R. Soc. Lond. B*, *272*: 181.
Gierer, A., and Meinhardt, H. (1972). *Kybernetik*, *12*: 30.
Inouye, K., and Takeuchi, I. (1980). *J. Cell Sci.*, *41*: 53.
Jermyn, K. A., Duffy, K. T. I., and Williams, J. G. (1989). *Nature*, *340*: 144.
Keener, J. P., and Tyson, J. J. (1986). *Physica D*, *21*: 307.
MacWilliams, H. K. (1982). In *Developmental Order: Its Origin and Regulation* (S. Subtelny and P. B. Green, eds.), Alan R. Liss, New York, pp. 463–483.
Matsukuma, S., and Durston, A. J. (1979). *J. Embryol. Exp. Morphol.*, *50*: 243.
Murray, J. D., Stanley, E. A., and Brown, D. L. (1986). *Proc. R. Soc. Lond. B*, *229*: 111.
Odell, G. M., and Bonner, J. T. (1986). *Philos. Trans. R. Soc. Lond. B*, *312*: 487.
Otte, A. P., Plomp, M. J. E., Arents, J. C., Janssens, P. M. W., and Van Driel, R. (1986). *Differentiation*, *32*: 185.
Pagola, A., Vidal, C., and Ross, J. (1988). *J. Phys. Chem.*, *92*: 163.
Primmett, D. R. N., Norris, W. E., Carlson, G. J., Keynes, R. J., and Stern, C. D. (1989). *Development*, *105*: 119.
Raper, K. B. (1940). *J. Elisha Mitchell Sci. Soc.*, *56*: 241.
Rubin, J., and Robertson, A. (1975). *J. Embryol. Exp. Morphol.*, *33*(1): 227.

Schaap, P., Van Lookeren Campagne, M. M., Van Driel, R. Spek, P., Van Haastert, P. J. M., and Pinas, J. (1986). *Dev. Biol.*, *118*: 52.
Schulman, L. S., and Seiden, P. E. (1986). *Science*, *233*: 425.
Siegert, F., and Weijer, C. J. (1989). *J. Cell Sci.*, *93*: 325.
Siegert, F., and Weijer, C. J. (1991). *Physica D*, *49*: 224.
Spemann, H. (1938). *Embryonic Development and Induction*, Yale University Press, New Haven, Conn. (reprinted in 1967 by Harper, New York).
Sternfeld, J., and David, C. N. (1981). *Differentiation*, *20*: 10.
Takeuchi, I., Kakutani, T., and Tasaka, M. (1988). *Dev. Genet.*, *9*: 607.
Tomchik, K., and Devreotes, P. (1981). *Science*, *212*: 443.
Varnum-Finney, B., Edwards, K. B., Voss, E., and Soll, D. R. (1987). *J. Cell Motil. Cytoskeleton*, *8*: 7.
Weijer, C. J., McDonald, S. A., and Durston, A. J. (1984). *Differentiation*, *28*: 9.
Williams, J. G. (1988). *Development*, *103*: 1.
Williams, K. L., Vardy, P. H., and Segel, L. A. (1986). *BioEssays*, *5*: 148.
Williams, J. G., Jermyn, K. A., and Duffy, K. T. (1989). *Development Suppl.*, 91.
Yamaguchi, T., and Müller, S. C. (1991). *Physica D*, *49*: 40.