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Morphogenesis in *Dictyostelium*: An Orbital Hypothesis

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Summary. Free-living amoebae of *Dictyostelium discoideum* aggregate when deprived of food, guided by the intercellular transmission of signals of adenosine 3′,5′-monophosphate. A succession of multicellular forms is then constructed, each with a circular cross section in every plane normal to its central axis. Amoebae are in constant circular and helical motion around the circumference of these structures. A theory is proposed wherein the sustained propagation of waves of cyclic adenosine 3′,5′-monophosphate secretion in cellular loops determines their circumference and thereby organizes morphogenesis in this organism.

Morphogenesis, the process by which cells are organized into tissue structures, is a central unsolved problem in biology. *Dictyostelium discoideum* provides a valuable system for its study. This cellular slime mold is found in nature on the forest floor, but can be readily cultured and studied on laboratory substrata such as agar (*1*).

The organism grows as independent amoebae until the exhaustion of food, typically bacteria, induces the cells to differentiate for aggregation into a series of characteristic multicellular forms. While considerable information about the biochemistry, intercellular communication, and behavior of amoebae during the aggregation stage is available (*2–5*), little is known about the determinants of the various multicellular structures, called grexes. We have recently observed that cells are constantly in motion, oriented circumferentially around the central axis of the grex, during this developmental period (*6, 7*). We propose a theory which interprets this orbital activity as being morphogenetic and directed by the same signaling mechanism that guided initial aggregation.

Aggregation Stage

Current behavioral and biochemical data support the following general scheme for slime mold aggregation. Morphogenesis typically commences several hours after the onset of starvation, when amoebae give up the constant searching motion characteristic of vegetative cells and begin oriented migration toward scattered centers that emit attractive signals with a regular periodicity (*3, 4, 8–10*). Extracellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP) appears to be the chemoattractant (*11*). Starving amoebae also develop the capacity to synthesize and secrete cyclic AMP when stimulated by extracellular cyclic AMP. This response, called relay, propagates the chemotactic signal from cell to cell over long distances (*3–5, 12*). Cells secrete cyclic AMP for a few minutes before adaptation to the stimulus temporarily suppresses further relay activity (*5*). Secreted and cell-surface phosphodiesterases then destroy the signal molecule, lowering the background and restoring cellular sensitivity to cyclic AMP in preparation for the next relay event (*2*).

Multicellular Grex Stage

As amoebae migrate into aggregation centers they become associated end-to-end in chains, which, in turn, condense into radial or spiral arboreal streams as shown in Fig. 1 (*9, 13*). Aggregates quickly become organized around a central axis that becomes a landmark in all the multicellular stages. Depending on conditions, the first grex to form may be a narrow papilla (*7*) or a broad hemispherical mound (Fig. 2A). The emergence of the apical papilla (Fig. 2B) heralds the elongation of the aggregate into a conical and then cylindrical column (Fig. 2C). The vertical column reclines to form a slug, which migrates within the slime sheath it continually secretes (Fig. 2D) (*14*). Upon finding a suitable environment—such as high light intensity or low humidity (*7, 15*)—the slug reestablishes a vertical axis. Its anterior portion points aloft while its posterior expands into a broad base. The grex thus comes to resemble a sombrero (Fig. 2E). The central cells elaborate a stalk, which carries aloft the peripheral cells as they become transformed into spores (*16*). The result is a fruiting body (Fig. 2F). When the spores become dispersed and find a suitable environment, they germinate to form amoebae which begin the life cycle anew.

Circumferential Motion in Grexes

We have observed by time-lapse cinemicrography the circumferential movement of cells at the surface of grexes at various stages of development (*6*). The motion of individual cells could be more easily followed in grexes of the mutant axenic strain, Ax-3, than in the wild-type strain, NC-4; accordingly, Ax-3 was used in most of our studies. Staining cells by feeding them the red bacterium *Serratia marcescens* or by treating them with neutral red also improved the visualization of individual cells. The overall motion in each organism was sustained rather than periodic and was uniformly

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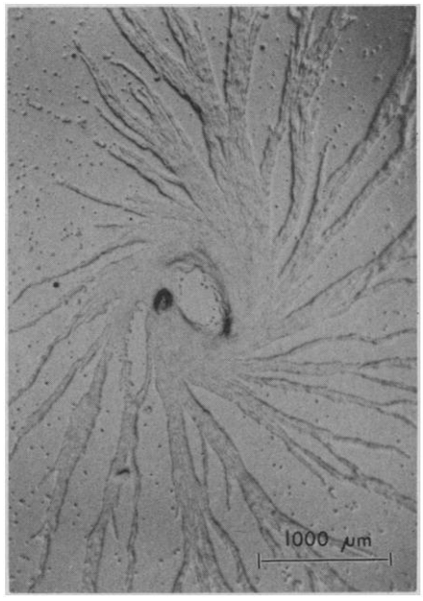


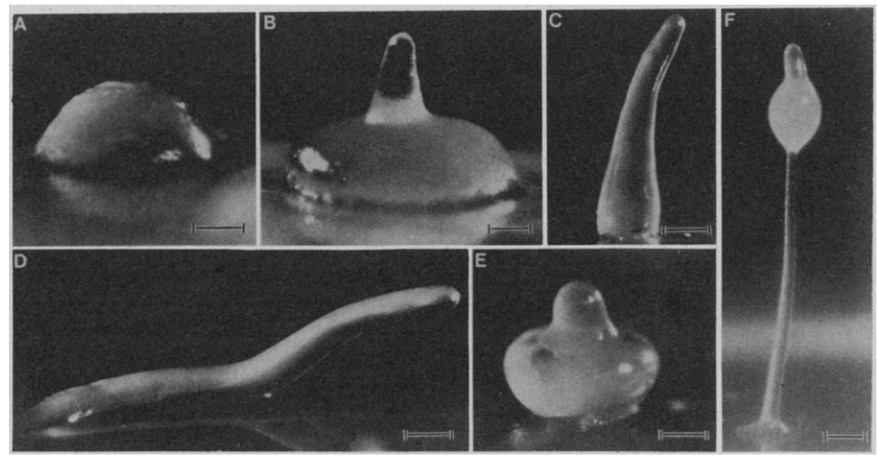


Fig. 1 (left). A spiral aggregation center. NC-4 amoebae have become organized into chains which have become associated into streams which have moved tangentially toward an attractive center created by a pacemaker loop of cells. Calibration bar, 100 μm. Fig. 2. (above). Major stages in the morphogenesis of *Dictyostelium discoideum*. (A) A mound; (B) a mound with a short column capped by a papilla; (C) a vertical column; (D) a slug; (E) a sombrero; and (F) a fruiting body. Each stage is organized by one (A, C, D) or two (B, E, F) dominant circumferences centered on a single axis. Calibration bars, 100 μm.

oriented, either clockwise or counterclockwise.

Mounds. Streams entered spiral centers as if wound on a spool. The growing aggregates rotated about their axes, apparently sustaining the movement of the converging streams. Under conditions where morphogenesis was delayed in the mound stage (*7*), circular cellular movement (at about 70 micrometers per minute) could be observed for hours in both the wild-type and mutant strains. The mounds also gyrated as a whole on the agar substratum. While the motion was best seen in time-lapse movies, we have documented the circumferential motion of cells in mounds by comparing single- and multiple-exposure photomicrographs (Fig. 3).

Papillae. When Ax-3 mounds formed papillae and elongated along their vertical axis, the cells spiraled from a wide orbit in the mound into a constricted one in the papilla without interruption of their regular movement. That is, the emergence and elaboration of the papilla and vertical column represented a transition in the circumference of circular motion.

Toruses. We have observed that, under defined conditions, mounds composed of mixtures of NC-4 and Ax-3 strains of *D. discoideum* broadened as a result of the spiral movement of their cells, first forming a central depression, then a hole, and finally a rotating torus (Fig. 4) (*7*). The velocity of circumferential movement was 37 ± 9 μm/min [standard deviation (S.D.)] (N = 6) and was independent of the size of the annulus. Eventually, toruses became interrupted by nodes at one or more sites. The cells downstream continued their motion around the torus and accumulated in these nodes (Fig. 4). The secondary aggregation centers that formed then completed normal morphogenesis. The transition from the mound to the torus demonstrates the expansion of a grex from one characteristic size to another via circumferential movement. Mound-to-column and mound-to-torus transformations suggest that orbital motion is morphogenetic and can produce either increases or decreases in grex circumference.

Slugs. We have also observed circumferential cellular motion in cinemicrographs of vitally stained migratory Ax-3 slugs. In this case, the movement was helical and directed anteriorly. The pitch was small (that is, the movement was nearly transverse). Helical movement was best seen in the front three-fourths of the slug, the tail showing primarily longitudinal migration.

An Orbital Theory of Morphogenesis

The study of morphogenesis seeks to identify the attributes of individual cells that determine their assembly into tissue structures. We suggest that *D. discoideum* regulates its morphology through the control of physiologic parameters governing sustained cell movement in response to intercellular cyclic AMP signaling. This process is taken to be a function of a flexible program of gene expression responsive to environmental variables.

Our argument begins with the observation that grex structures can be idealized as simple shapes, such as the hemispherical mound, conical and cylindrical columns and slugs, the toroidal brim of the sombrero, and the ellipsoidal spore mass (Fig. 2). All these forms have a circular transverse cross section at every level. The contour of a grex is therefore described by the set of radii normal to its central axis. How are these radii physiologically specified?

In principle, a loop of cells can relay a cyclic AMP signal as a traveling wave in a perpetual cycle. The mechanism of adaptation mentioned above would prevent the signal from being relayed back in the direction from which it came, thus assuring its unidirectional propagation (*17*). Cells in such loops would also respond chemotactically, moving circumferentially in the direction opposite to the cyclic AMP wave front. Nearby cells would likewise be drawn toward this signaling structure. In a loop of circumference C (micrometers) bearing a wave of velocity V (micrometers per minute), the interval for one orbit, I (minutes), is given by the expression

$$C = I \cdot V \quad (1)$$

It is a reasonable simplification to assume that the cyclic AMP relay in *D. discoideum* consists of cycles of secretion and refractoriness with an overall period of P (minutes). To sustain an orbiting wave, a loop of cells must be large enough to allow time for recovery of relay sensitivity (that is, "deadaptation") between wave fronts. Specifically, an orbiting wave can be sustained only in loops where $I \geq P$ or

$$C \geq P \cdot V \quad (2)$$

We believe with Durston (*18*) that loops in which I exceeds P will tend to diminish in size until $I = P$. Two mechanisms might contribute to this process. First, when I is greater than P, each cell in a loop will become sensitive to cyclic AMP signals before the wave front returns. During the $(I - P)$ hiatus, these cells can be recruited by other signaling centers. As cells leave the loop, I will approach P until, at $I = P$, the depletion ceases. Second, relay activity in the loop emits cyclic AMP in all directions, thus stimulating cells closer to the loop's center. These cells can themselves form loops of smaller I. Ultimately, a minimal loop will evolve in which $I = P$. Because of the higher angular velocity of its wave front, the minimal loop will attract and entrain the more distal cells. The population will thus approach a stable circumference:

$$C = P \cdot V \quad (3)$$

Equation 3 is a useful expression, since it provides a mechanistic link between cellular properties and grex morphology. That is, the circumferences of grex structures can be controlled by the P and V values of cells in loops satisfying Eq. 3. We call these structures $P{\cdot}V$ loops.

Evidence for $P \cdot V$ Loops

Grex cells respond to cyclic AMP. Whole slugs (*19*) and dissected slug tips (*20*) are attractive to chemotactically competent amoebae. Whole slugs attract dispersed slug cells (*21*). The attractant is likely to be cyclic AMP (*22*), since cells dispersed from slugs respond chemotactically to cyclic AMP (*21*), slugs contain an appreciable amount of both extracellular and intracellular cyclic AMP (*23*), slugs are perturbed by exogenous cyclic AMP (*24*), and preparations of intact slugs and cells dispersed therefrom are competent to relay cyclic AMP (*25*).

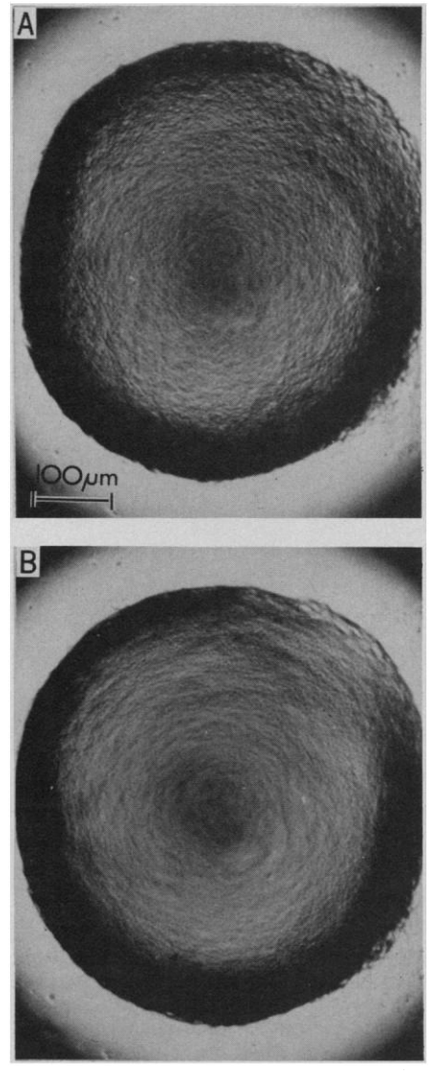
Loops in spiral aggregates. The relay of cyclic AMP through fields of aggregating amoebae appears to be accompanied by a change in the light-scattering properties of the cells, making this activity visible in favorable time-lapse cinemicrographs as broad bands spreading centrifugally from pacemaker centers with regular periodicity (*3, 4*). The waves of relay are propagated radially as concentric rings when they emanate from a point source and in a spiral pattern when the signaling center is a pacemaker loop (*3, 4, 18*).

Spiral propagation of chemoattractant elicits spiral aggregation, as in Fig. 1. The regular, uninterrupted orbit seen in the relayed wave front tells us that it is the product of a cycling wave rather than an autonomous pacemaker locus within the loop. We therefore conclude that the propagation of attractant in loops is morphogenetic at least at this stage of development.

Relation of C *to* P *and* V *in grexes.* Whether $C = P \cdot V$ in either spiral centers or grexes is not known. P and V have not been measured in grexes; however, they can be monitored during early aggregation. The interval between successive relayed waves may be taken as an upper bound for P in both radial and spiral centers (*3, 4, 10*) and in experiments where cyclic AMP pulses are delivered periodically from a microelectrode (*26*). These data suggest that P varies between 2 and 10 minutes.

The wave velocity, V, was 40 to 500 μm/min in a variety of aggregation territories (*3, 4, 17, 26*). Multiplying the extreme values of P and V, we predict a range for C of 80 to 5000 μm. The circumferences we have measured for various grex structures range from an average of 190 μm for NC-4 papillae (for example, Fig. 2B) to 4200 μm for the largest torus formed by mixtures of NC-4 and Ax-3 cells (for example, Fig. 4). We conclude that Eq. 3 is not inconsistent with the available data.

Contributing to the dispersion in the P and V values given above is the fall in the value of both parameters with time during aggregation (*4, 10*). The circumferences of aggregates also diminish during early morphogenesis, suggesting that P or V (or both) may continue to decline in the grex. For example, the circumference of a central loop in roughly 10-hour spiral aggregates of NC-4 cells measured 1390 ± 890 μm (S.D.) (N = 12); the widest circumference of the vertical column (typically, at 17 hours of development) was 530 ± 270 μm (N = 38); and the greatest girth of migrating slugs (at an average age of 22 hours) was 305 ± 94 μm (N = 50).



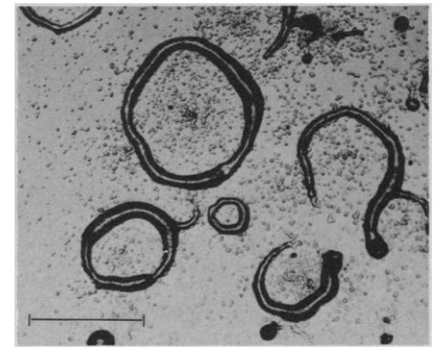


Fig. 3 (left). Circumferential movements in a mound. A mound of NC-4 cells developed for 19 hours at 15°C on 1.25-mm thick 1 percent agar was photographed under dark-field illumination. (A) A single 8-second exposure. (B) Four superimposed 4-second exposures, made 20 seconds apart, over a total period of 64 seconds. The discrete circular patterns seen in panel A are circumferentially streaked in panel B, signifying orbital motion. Calibration bar, 100 μm. Fig. 4 (above). Toruses. A mixture (1:3) of NC-4 and Ax-3 cells was allowed to develop for 17 hours as described (*7*). Some of the toruses that formed have broken and are condensing into mounds by circumferential cell migration. Calibration bar, 1000 μm.

Multiplicity of $P \cdot V$ Loops

One can imagine that there are many $P \cdot V$ loops in every plane normal to the axis of a grex. A sufficient dispersion in values of P or V, or both, would allow most of the cells in any cross section to participate in a set of concentric $P \cdot V$ loops. However, there would probably always be an axial core too small to sustain traveling waves, leading to relative inactivity of cells at the center.

Variations in P and V could also generate continuous gradations in the contour along the axis of the grex (Fig. 2, A, C, and D). Some grex forms are compound, however, with an abrupt change in curvature demarcating the component structures (Fig. 2, B, E, and F). Values of P and V expressed by the cells in such grexes might fall into two classes that impose the observed differences in circumference. A conceivable mechanism for the segregation of the two cell types is that chemotactic sensitivity has a periodicity and phase matching that of relay. Cells would then move preferentially toward the periodic signal they most often perceived. Tissue proportionation would reflect the relative preponderance of cells in each class of periodicity. An extreme example of such behavior might be the binary grexes sometimes elaborated by mixtures of strains NC-4 and Ax-3 (*7*). Here, mixed mounds segregate into two independent grexes of broad and narrow circumference (Fig. 5).

Alternatively, there may be only a small number of $P \cdot V$ loops in a grex, to which the remaining cells respond chemotactically in assuming their position. A precedent for this possibility is provided by the spiral aggregation center where a single loop determines the disposition of large numbers of cells in its vicinity (Fig. 1). Rare loops stationed along the axis of a grex could define its one or two principal circumferences, thus acting as classical organizers (*27*).

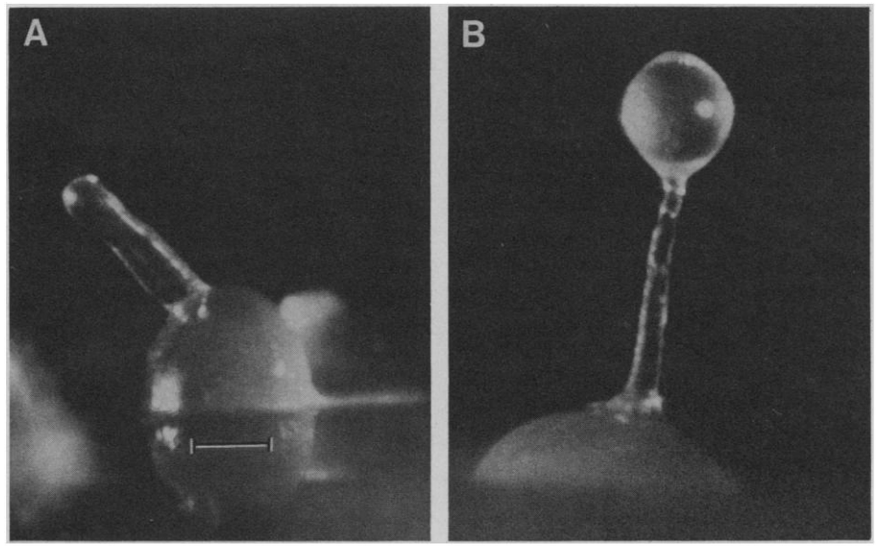


Fig. 5. Binary grexes. A mixture (1:1) of NC-4 and Ax-3 was allowed to develop on a Millipore filter substratum as described (*7*) for (A) 15 hours and (B) 20 hours. The narrow central structures appear to be predominantly NC-4 (*7*). This result suggests that the specification of tissue circumferences is a stable and characteristic trait of each of these closely related strains. Calibration bar, 50 μm.

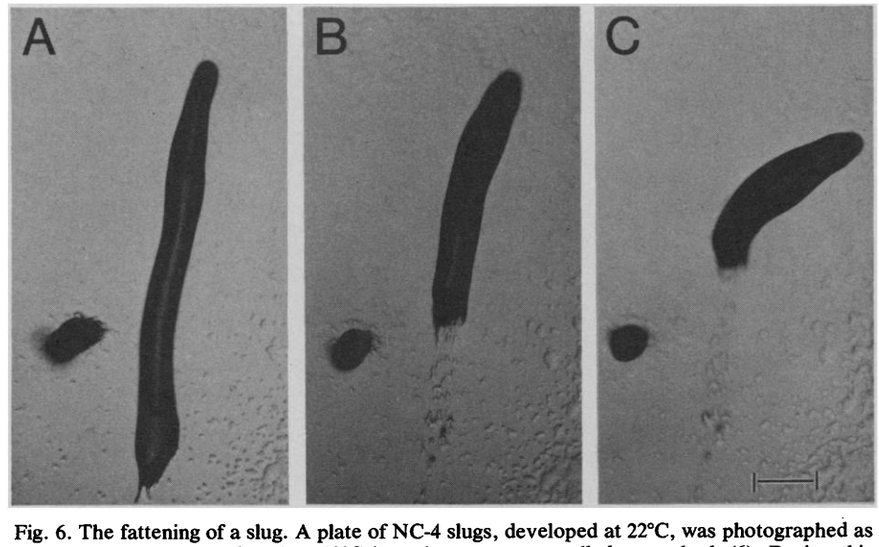


Fig. 6. The fattening of a slug. A plate of NC-4 slugs, developed at 22°C, was photographed as the temperature was reduced to 12°C in a thermostat-controlled water bath (*6*). During this period, many slugs shortened and broadened. (The stationary mass on the left of the slug shown here is a useful reference point.) We suggest that the change in proportions could be the result of a reduction in the pitch of the helical path of cell movement starting at the anterior pole (see text). Calibration bar, 200 μm.

$P \cdot V$ Loops as Organizers

Time-lapse cinemicrographs of papilla formation have provided the strongest evidence for singular $P \cdot V$ loops acting as organizers through the recruitment of neighboring cells. The papilla is seen to emerge abruptly as a vortex, which draws cells from the underlying mound into its narrow orbit. The erection of the vertical column is an immediate consequence of the continued spiral influx of cells (Fig. 2B).

We thus hypothesize that elongated structures such as vertical columns and slugs are shaped by a single $P \cdot V$ loop. But how does this organizer govern the circumference of the subjacent column of cells? The fact that the cells on the slug surface spiral anteriorly suggests that the apex is the source of an orbiting wave front of cyclic AMP. The resemblance of the helical motion in the slug to the spiral movement of cells aggregating toward a pacemaker loop (as in Fig. 1) suggests an analogy; namely, the slug behaves as a spiral aggregation center applied to a cylindrical rather than a planar surface. We think that it could be the transmission of the orbiting cyclic AMP wave front helically down the slug that controls its circumference.

The idea of an apical organizer presents other interesting implications. The same mechanism by which this narrow loop would effect the ascent of cells into columns could chemotactically guide their subsequent horizontal translation as well. Thus, the $P \cdot V$ loop responsible for shaping the slug could subsequently excite its migration. Furthermore, the pitch of the helix relates the linear velocity of cell movement to the axial velocity

of the migrating slug itself: a slug whose cells spiral with a great pitch (that is, longitudinal orientation) would migrate more rapidly than a slug with a shallow pitch (that is, transverse orientation).

In this regard, we have observed in time-lapse cinemicrographs that a rapid shift in temperature from 22° to 12°C caused slugs to slow [see also (*28*)] and to fatten (Fig. 6). Thus, both the change in proportions and velocity could reflect a reduction in the pitch of the helical migration path of cells along the slug. This change presumably started at the apex, since the forward motion of anterior pole of the slug slowed relative to the posterior pole (Fig. 6). Such an increase in circumference would be expected from our model if temperature influences P and V in slugs as it does during aggregation; that is, $P \cdot V$ diminishes with increasing temperature (*4*).

Finally, the helical movement of cells along the axis of the slug encourages an explanation for an obscure phenomenon originally reported by Francis (*28*). Vertical columns are observed to recline to the horizontal by slow helical gyrations, from apex to base, around their long axis (Fig. 7). If the cells in the slug were organized as a cluster of nested helices, like strands in a rope, then the observed flexion could reflect the coiling of these coils, perhaps attributable to the "supertwisting" of strands about the slug axis.

Concluding Comments

Major events in the morphogenesis of *D. discoideum* can be appreciated in terms of the orbital hypothesis, since they involve programmed shifts in the circumferences of grex structures. The center of spiral aggregates would obviously contain the first morphogenetic loop observed during development. Hemispherical mounds would later be organized by broad $P \cdot V$ loops, perhaps preserving those of the spiral centers. The P and V values expressed during the early multicellular stage would be adjustable so as to bring the cells into close packing despite wide variation in the size of the grexes. Eventually, small loops emerge with a specific morphogenetic function: to impose a narrow and elongated contour on the young grex. Finally, the expansion of the posterior pole of the slug to form the brim of the sombrero would be effected by broad $P \cdot V$ loops.

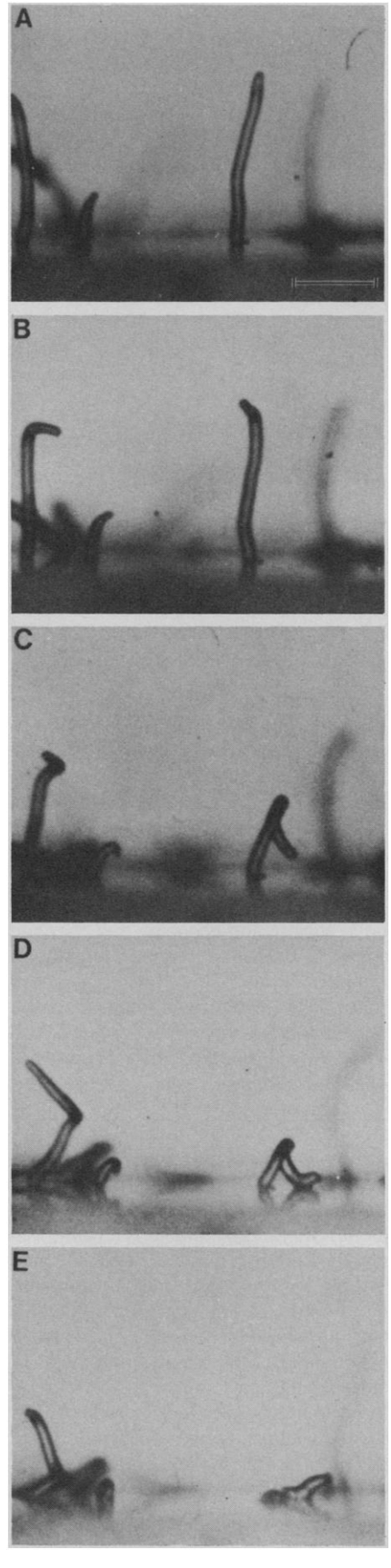


Fig. 7. Supercoiled slugs. Vertical columns of NC-4 cells formed after 16 hours of development were photographed as they reclined by time-lapse cinemicrography from the side. The temporal relationship of the frames shown here is (A) 0 minutes, (B) 7 minutes, (C) 18 minutes, (D) 25 minutes, and (E) 33 minutes. Note the coiling and rotation of the two slugs in focus. Calibration bar, 500 μm.

The same loops that controlled the erection of the slug might be retained in the crown of the sombrero to guide the lengthening of the slim column of stalk cell precursors. The number of $P \cdot V$ loops in a grex is unknown but could be many or few.

Two abnormalities in the morphogenesis of grexes composed of mixtures of strains NC-4 and Ax-3 can also be understood in terms of $P \cdot V$ loop expression. First, the expansion of composite mounds into toruses (Fig. 4), which always occurs during a period in which no papilla (that is, narrowing organizer) is manifested, could reflect the unbalanced expression of broad $P \cdot V$ loops. Second, the formation of binary grexes composed of a narrow slug sprouted from a broad mound (Fig. 5) could represent the concurrent but noncoordinated expression of two $P \cdot V$ loop programs.

In conclusion, while most theories of morphogenesis postulate the movement of cells, they also stipulate that net motion ceases once the cells are correctly positioned. In contrast, sustained cellular motion is integral to the morphogenetic mechanism we propose. The requirement for orbital paths may restrict the wider application of our hypothesis since many complex tissue structures lack such simple symmetry. However, circular cross sections are seen elsewhere in ontogeny and phylogeny; their origin might profitably be investigated in terms of this developmental model for *D. discoideum* (*29*)

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30. We thank P. N. Devreotes and M. C. Dinauer for discussions and K. J. Tomchik and G. S. Retzinger for technical assistance. This work was supported by grant GM 22321 from the Public Health Service, a postdoctoral fellowship from the Anna Fuller Fund (to R.L.C.), and a Faculty Research Award from the American Cancer Society to T.L.S.

Forests in the Long Sweep of American History

Marion Clawson

The current concern over forest policy, including policy with respect to the national forests, focuses on the recent past, the present, and the relatively near future to a degree that threatens to obscure longer and more basic trends in American forestry. To coin a phrase, the trees are obscuring the forest. Forestry is a long-term matter, as foresters and many others have long emphasized, but all too often the longer trends are overlooked or underestimated. A knowledge and understanding of the past is essential to sound public and private decisions on forest land use, forest investment, timber harvest, and other forest uses.

A quantitative history of forest land use, timber stands, timber growth, and timber harvest from 1800 to date, as presented in this article, is, like many other histories, limited by the paucity, suspected inaccuracy, and noncomparability of the available data (*1*). The best available data have been used in this article and are sufficient to sustain the interpretations drawn.

Summary. The role of forests in the American society and economy has changed greatly over the past 200 years, as is evidenced by data on acreage in forests, on volume of standing timber, on amount of annual wood growth, on amounts and form of wood utilization, and on prices for forest products. The two most dramatic facts in a long history of forest utilization have been the near fourfold increase in annual wood growth in the past 60 years and the persistent and major underestimate by the U.S. Forest Service of the wood production potential of American forests.

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Forests as a Land Use

Forests have always been important in the American economy and way of life. Ready building material and fuel were important to early pioneers even when much of the forest had a negative value and the land had to be cleared for cropping. As the nation expanded westward, the lumber from the forests provided a major share of the building materials. In more recent times, the forests have come to be more appreciated for their watershed, wildlife, recreation, wilderness, and esthetic values. There is good reason to believe that the role of forests in American life will increase in importance in the next several decades.

After a long and substantial decline in forest land area from 1800 onward, forest area has been approximately stabilized since 1920 (Fig. 1 and Table 1).

"When the explorers landed, America was trees . . . explorers looked down from the mountain tops to an ocean of trees that stretched in every direction as far as the eye could reach . . . filled them with awe. They felt besieged by the poignant immensity, the wild monotony" (*2*). In these words Lillard conveys at least some of the early reactions to the forests of the East. The early Spanish explorers coming into the Southwest from Mexico were overwhelmed, not by the forests, but by the immensity of the plains and deserts.

There is a fair amount of agreement as to the original forested areas of the United States (*3*). Using maps showing natural forested areas, workers have estimated the area of what today we call (erroneously) "commercial forest" at about 850 million acres at the time of the earliest colonization. While there was some land clearing before 1800, it was modest indeed. The original area of what today we call noncommercial forest is likewise estimated at about 100 million acres. I cannot but wonder if that was much too low, but the point is not important for the discussion in this article. These figures should be compared with the 1904 million acres of all land in the 48 contiguous states; roughly half of the area was originally forested.

Land clearing for farm cropland and improved pasture was comparatively small until the mid-19th century (Table 2). Thereafter, it proceeded more rapidly. Cropland area reached a high plateau in the interwar period of the 1920's and 1930's, but much of the increase in cropland area after about 1900 was in the Great Plains, a normally nearly treeless region. In 1909 Greeley (*4*) presented fig-

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