I. INTRODUCTION

The cellular slime mold *Dictyostelium discoideum* is a mononucleate soil amoeba which feeds and divides as a unicellular organism. On a solid substratum, after the food supply is exhausted, the amoebas begin multicellular morphogenesis [1] (Fig. 1). After an 8-h interphase, the amoebas collect into hemispherical mounds fed by radial, randomly bifurcating streams of cells (aggregation stage). Each aggregate secretes an external slime sheath, containing mucopolysaccharide and protein, which is elastic at the apex and rigid at the periphery of the aggregate. Incoming cells are thus constrained to enter a vertical, approximately cylindrical

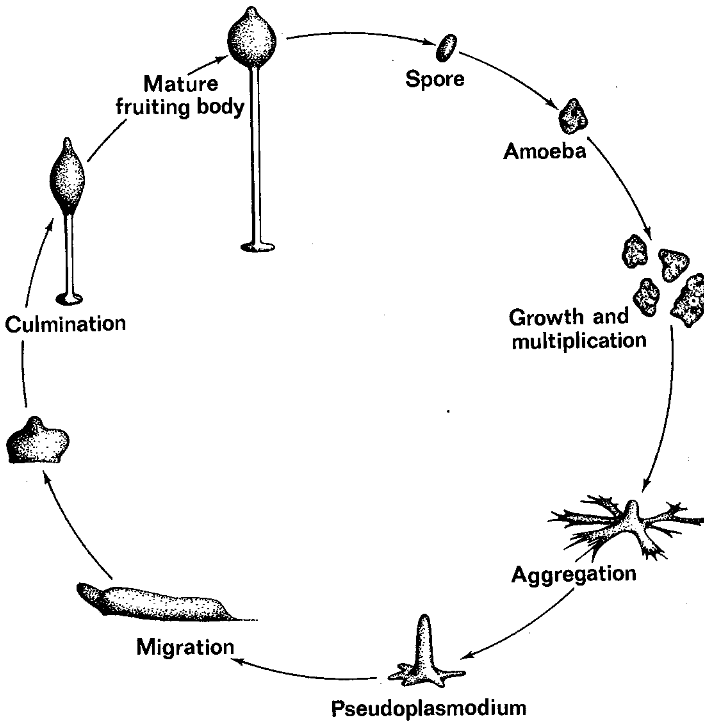


FIG. 1. The life cycle of the cellular slime mold *Dictyostelium discoideum*.

structure, producing the pseudoplasmodium or slug stage. The slug falls over and migrates for a variable time, leaving behind a trail of collapsed slime sheath, before it finally rounds up and constructs a fruiting body (culmination stage). A vertical cellulosic tube (stalk) is now secreted within the culminating slug and is extended by addition to its top end. Some cells enter the stalk, vacuolate, and die (stalk cells). Most of the others are raised on top of the elongating stalk and secrete a thick cell wall to become viable spores. A few cells which were originally at the rear of the slug are left at the base of the stalk and make a characteristic basal disk of dead, vacuolated cells. The pattern of cell types in the fruiting body is approximately proportionate over the 10^4-fold volume range of the organism (sorocarps contain 10 to 10^5 cells) [2]. It originates in a regulative axial pattern of embryonic cell types in the migrating slug [3].

The *Dictyostelium* life cycle is of great interest for developmental biologists because it poses, in elementally simple form, the problem of how an organism is assembled from component cells during embryogenesis. In the following section, I discuss investigations of the mechanisms of multicellular pattern formation in the development of *D. discoideum*.

II. CURRENT RESEARCH

A. The Control of Aggregation

Because aggregation occurs through the coordinated movement of a two-dimensional array of initially separate cells, much can be learned about its control by direct observation using time-lapse cinemicrography. Time-lapse films of aggregation reveal beautiful and complex spatio-temporal movement patterns [1,4-6]. The individual cells approach centers by making pulsatile movements. These movements are a result of directional pseudopodial extensions. The cells move about 20 μm in 100 sec. The movements are repeated rhythmically by each cell (once every 2 to 10 min), and movements

of individual cells are coordinated in space and time, so that periodic waves of (centipetal) cell movements propagate centrifugally from each aggregation center at constant or increasing velocities in the order of 50 μm/min. When waves from neighboring centers collide they are mutually annihilated and thus define boundaries between domains of influence for the respective centers. Visible wave propagation continues until the end of aggregation.

The late aggregate forms an apical nipple of cells (the tip), which is the origin for any later waves. The tip persists as a distinct structure in later morphogenesis [at the front of the migrating slug and at the apex of the developing fruiting body (Fig. 1)] and it is of great importance. It has proved to act as an embryonic organizer (morphogenetic boundary region) for all stages of development in which it is present [6-8]. The above observations are evidence for a complex control system regulating movement during morphogenesis.

There is clear evidence that cells of *D. discoideum* aggregate by chemotaxis to cyclic adenosine-3',5'-monophosphate (cAMP) [9-11]. Aggregating cells are also known to secrete a soluble cAMP phosphodiesterase (soluble PDE) [12] as well as a PDE inhibitor [13] and to have a cell-bound PDE [14] as well as a surface-bound nonenzymatic cAMP binding protein [15,16]. Of the above, PDE inhibitor, cell-bound PDE, and the cAMP-binding protein (as well as cAMP secretion and chemotactic sensitivity) are aggregation stage-specific and are not seen in vegetative cells. The functions of the cAMP-related proteins are unknown, but there is reason to suspect that the nonenzymatic binding protein may be the chemotactic receptor (see below).

A number of tests have been used to measure the chemotactic sensitivity of *D. discoideum* cells to cAMP [11,17-20]. In the most sophisticated of these [18,19], calibrated pulses of cAMP are delivered locally to a monolayer of cells on an agar surface by electrophoresis using a glass microelectrode with an internal diameter of about 2 μm. The pulses diffuse symmetrically from the electrode tip and are depleted at a calculable rate by the PDE enzymes to

give standard spatio-temporal cAMP profiles. The test shows, in agreement with a previous result [11], that the cells of *D. discoideum* manifest full chemotactic sensitivity at 4 h after starvation. They are then attracted from within a defined radius of the electrode tip, the radius depending on the pulse size. This suggests that there may be a threshold signal for chemotaxis (i.e., that the chemotactic response is highly nonlinear). If calculated as the maximum cAMP concentration reached at the chemotactic radius following a cAMP pulse, the threshold corresponds to between 1 and 4×10^{-9} M cAMP (based on standard pulse amplitudes of 6×10^{9} and 6×10^{10} molecules). This is in agreement with an independent result for the minimum effective cAMP concentration for chemotaxis [17]. It is very similar to the dissociation constant of the nonenzymatic cAMP binding protein, supporting the contention that this may be the chemotactic receptor [15]. The PDE enzymes have higher Km values [12,14]. At this time, we know nothing more about the quantitative dependence of the chemotactic response on the perceived signal nor anything about the nature of the perceived signal (whether cells detect a space or a time gradient of cAMP). A quantitative analysis, using appropriate test environments (e.g., stationary space gradients of cAMP), is required.

Bonner [22] made an important observation about the chemotactic response, namely, that chemotactically responsive cells have differentiated to a polar state. Vegetative (nonchemotactic) cells are isotropic and move by extruding characteristic pseudopods [23, 24] from randomly situated points around the cell periphery. Chemotactically responding cells, observed after the beginning of aggregation, make pseudopods only from about the front third of the cell surface, and the pseudopods are induced synchronously among the local cell population by chemotactic signals. This polarity is fixed, since cells switching allegiance between aggregation centers 180° apart usually preserve polarity and make "U turns" [22]. It does not account for the directional nature of chemotaxis, since cells make signal-directed pseudopods from the permitted part of the cell surface and hence can make "U turns." It does appear

necessary for directional chemotaxis during natural aggregation signals, since I have isolated a mutant [10] which lacks normal polarity and consequently fails to aggregate. This mutant makes spatio-temporal waves of cell movements which are normal except that the responding cells fail to make a net centripetal movement with each wave. Instead, the cells make mainly forward pseudopods as each wave approaches and backward pseudopods as it passes. Thus they move forward and then return to their original positions. As discussed later, polarity appears to be required if cells are to be refractory to retrograde stimulation by the natural chemotactic waves of secreted cAMP.

Polarity appears to develop during the course of aggregation. Cells responding to the first few aggregation waves make synchronous center-directed movement steps in response to the waves and randomly timed and directed movements between waves. The latter occur by apolar pseudopod formation from any part of the cell surface. As aggregation proceeds, apolar movements gradually cease and the only movements are polar and synchronous in response to chemotactic signals. This gradual polarization appears to be initiated by perception of chemotactic signals, since cells are capable of making polar chemotactic movements as early as 4 h before the onset of aggregation if they are signaled appropriately. It may represent a second polar response of cells to cAMP other than directional pseudopod formation. The frequency of pseudopod formation decreases as polarity develops, suggesting that cells become generally more refractory for pseudopod formation while also developing an antero-posterior gradient of refractivity.

B. Signal Relaying during Aggregation

The nondecremental waves of movement seen during aggregation are not expected from a chemotactic response to cAMP signals diffusing from aggregation centers. Signal amplitude and velocity (i.e., velocity of cAMP concentration contours) should diminish

rapidly with distance from the center because of diffusion and extracellular PDE activity. The waves imply a signal-relaying competence: that cells sensing a chemotactic signal are simultaneously induced to make a new one, so that a local signal initiates a propagating wave of cAMP secretion [25]. The simplest type of signal-relaying in response to a cAMP signal was indicated by the finding that cAMP pulses applied locally to an appropriate aggregation field can initiate typical aggregation waves [18]. It has now been verified by the finding that pulses of cAMP can induce secretion of pulses of tritiated cAMP ([^{3}H]cAMP) by a labeled population of *D. discoideum* cells [26,27].

Little more is known about the parameters or mechanism of the relaying response except the following:

1. Wave initiation by cAMP pulses is pulse amplitude-dependent in an all or nothing fashion, indicating that there may be a threshold signal for signal relaying. Calculated as a concentration, this is 10^{-7} M [19]. No detailed quantitative results are available from the pulse amplification ([^{3}H]cAMP) experiments, but these give a positive response with concentrations as low as 6×10^{-8} M [27].
2. The low velocity of aggregation waves indicates that they are not rate-limited by diffusion from cell to cell and that there is a delay of the order of 15 sec between signal sensing and signal secretion in each cell [28]. The pulse amplification experiments show a rise time of 90 sec between the applied cAMP pulse and the [^{3}H]cAMP peak, but a cascade mechanism (among the cell population) has not been eliminated as a contributing factor.
3. The unidirectional nature of wave propagation and annihilation of colliding waves imply that each wave front is succeeded by a zone of refractory cells which are unable to relay the signal. Cells must therefore enter a refractory period after relaying a signal. This deduction has been confirmed by use of cAMP microelectrodes. It is found that high-frequency (1/3 min) cAMP pulses may be gated, with only every second or third pulse

initiating a wave. The refractory period is a monotonically decreasing, time-dependent variable, equal to 9 min at the beginning and 2.5 min at the end of aggregation [6,19].

4. Fields of slime mold cells will not propagate waves at a density of less than 2.5×10^4 cells/cm^2. (At this density, an average cell has just enough randomly distributed, competent neighbors in range (i.e., anterior and posterior neighbors in a connected chain) [28-30]. The critical mean number of neighbors is 4.5, giving a range of 75 μm for the observed critical density (2.5×10^4 cells) [29]. This range can be used to calculate signal amplitude (given a realistic value for PDE degradation of cAMP, hemispherical diffusion with known rate of diffusion through the agar substrate, and a known threshold for a signal relaying) [19, 28]. Such a calculation has been made, assuming a maximally efficient durationless (δ function) signal. The calculation gives a value of 1×10^8 cAMP molecules/cell/signal [19].

   The amplification experiments quoted above have also been used for direct measurement of signal amplitude and give a lower value (mean = 6×10^6 molecules/cell/signal) [27]. They also give data on the form of the cAMP signal secreted by a population (a pulse ∿90 sec wide) [26,27]. At this time, the form and amplitude of the signal secreted by a single cell are in doubt. If Robertson's [19] calculations are reliable and if the amount of cAMP secreted per cell does not exceed his calculated value, as suggested by the amplication experiments [27], *then the relayed signal must be a very short pulse* (duration ≤ a few seconds) and the 90-sec peak width seen in amplification experiments must be an artifact due to variable delay times in individual cells [28]. A signal lasting 90 sec would require several orders of magnitude more cAMP/signal to reach the same range as a (maximally efficient) durationless pulse.

5. Coalescence of aggregating cells into radial streams is a consequence of signal relaying. Because all cells are local repeaters of the signal, aggregating cells are attracted towards their nearest anterior neighbors and azimuthal maxima in cell density grow [29].

Once they enter aggregation streams, cells make ethylenediamine tetraacetic acid (EDTA)-resistant polar contacts (the signal-sensitive front end of each cell adheres to the back of a preceding cell) [4,31]. These contacts probably serve a synaptic function (i.e., now mediate signal propagation between cells) because cells joining streams are attracted towards the junctions between cells [25]. It is noticeable that large contractile vacuoles (∿3% of the cell volume) which were expelled at random points on the periphery of the vegetative amoeba are now expelled at the rear of each polar cell, apparently into its junction with the next cell. It is conceivable that these vacuoles play a role in signal secretion, though their frequency (∿1/30 sec) far exceeds the signal frequency.

The formation of synaptic contacts would be expected to change the nature of the aggregation field. In early aggregation, where the separate cells communicate by (symmetrical) diffusion through extracellular space, wave propagation is isotropic (i.e., possible with any orientation). Restriction of signaling to junctions would make each aggregate locally anisotropic, i.e., signaling would be possible away from but not toward aggregation centers [32]. There is experimental evidence that this is indeed the case [24,32].

The intercellular contacts made during aggregation are long-lived. I have observed that individual junctions last for more than 20 min (the length of the observation period). They may, in fact, persist into the slug (since strings of connected cells can be recovered from slugs) [4].

C. Autonomous Signaling during Aggregation

In addition to signal relaying, we must infer that some cells make autonomous signals and so spark off new waves of signal secretion. Two possibilities are conceivable:

1. Signal relaying and autonomous signaling are two aspects of the same property (periodic secretion of cAMP pulses due to an os-

cillator which can be driven faster than its natural frequency by external signals). Individual cells with the highest natural frequencies act as pacemaker centers and entrain others.

2. Autonomous signaling and signal relaying are separate properties, not necessarily present simultaneously in each cell.

There is clear evidence for item 2, both from periodicity measurements of aggregation waves as will be described and from measurements of emergence of signal-relaying competence (X2) [33] and of autonomous signaling competence (X3) [34,35]. X2(t) is determined by measuring the time, as a function of cell density, when a cell population can just propagate a wave of signaling in response to an applied signal from a microelectrode. This gives the time when the population contains a critical density of relaying-competent cells, and hence gives X2(t), which is the fraction of relaying-competent cells as a function of time. Critical density itself varies with cell density because PDE dissipation of the signal varies with density. It is determined as a function of cell density by diluting relaying-competent, mature, wild-type cells with a relaying-deficient but otherwise normal mutant. X2 is an S-shaped function with the first few cells gaining competence at 400 min, most cells at 500 min, and all cells by 600 min after starvation. X3(t) is measured by recording times of initiation of aggregation in uniform, small populations of cells, containing about 500 cells in a high-cell density drop on an agar surface. In such populations, aggregation begins well after all cells have acquired the chemotactic and signal-relaying competences. It is initiated by emergence of autonomous cells. If we assume independent emergence of autonomy in each cell we can calculate the mean number of autonomous cells per drop as a function of time and hence X3(t). The fraction of unaggregated drops at any one time is then the zero term of a poisson series. On this assumption, X3 is approximately linear, crossing the origin at 500 min and increasing at about 10^{-3} cells/h. Virtually all aggregating cells relay signals but very few can make spontaneous signals.

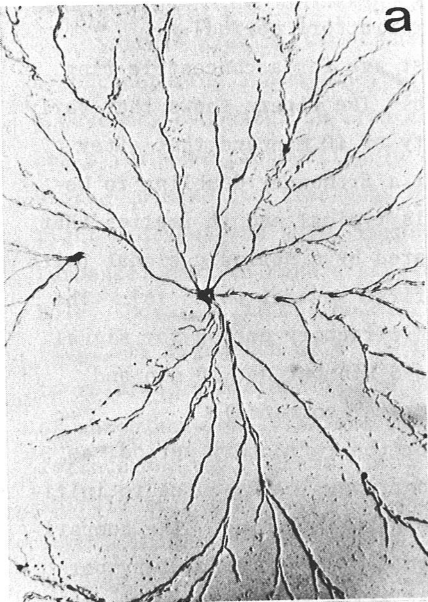
D. Signal Periodicity in Aggregation Waves

Much can be learned about autonomous signaling and the roles of autonomous signaling and signal relaying in aggregation from measuring the periodicity of aggregation waves. The waves are of the two geometrical types expected in a two-dimensional, isotropic-sensitive medium. They are either expanding rings, initiated at points, or expanding spirals, with an inner free end rotating about a central core [32,36].

A ring is the wave form expected from a localized, spontaneous signal (as from an autonomous cell), which should spark off a symmetrical wave of signal relaying among neighboring cells. Rings are the most common wave form and are the first waves seen. They usually occur as sequences of concentric rings, each initiated at the same point, indicating existence of continuously or continually signaling autonomous sources. These concentric waves organize aggregates with radial streams, as shown in Fig. 2a.

The periodicity of the waves should reveal the nature of the autonomous source. If the source makes a continuous superthreshold signal, successive waves will be separated by the refractory period for signal relaying (i.e., by the minimum possible interval). If the source makes intermittent superthreshold signals, then each signal will initiate a wave only if the signal interval exceeds the

FIG. 2 (opposite). Geometries of aggregates and periodicity of aggregation waves. (a) Concentric ring waves organize aggregates with radial streams, as in this example. The long axes of major streams are approximately perpendicular to the wave fronts and each stream propagates a segment of each wave. (b) Periodicity of waves from a concentric ring wave aggregation center. The ordinate records temporal intervals (minutes) between successive waves. The abscissa shows age of the center (minutes from starvation). The time of tip formation is indicated with an arrow. (c) Spiral waves organize aggregates with spiral aggregation streams. (The streams rotate counter to the spiral wave.) This aggregate has an open center which is circumvented by the inner end of the spiral wave. (d) Periodicity of a spiral aggregation wave. The ordinate shows temporal interval (minutes) between successive coils of the spiral wave. The abscissa shows age of the spiral (minutes from starvation).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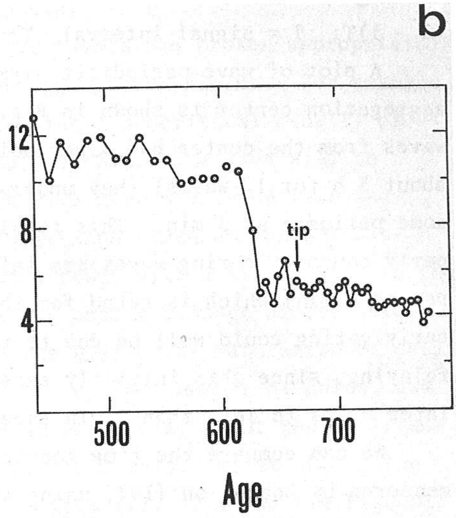
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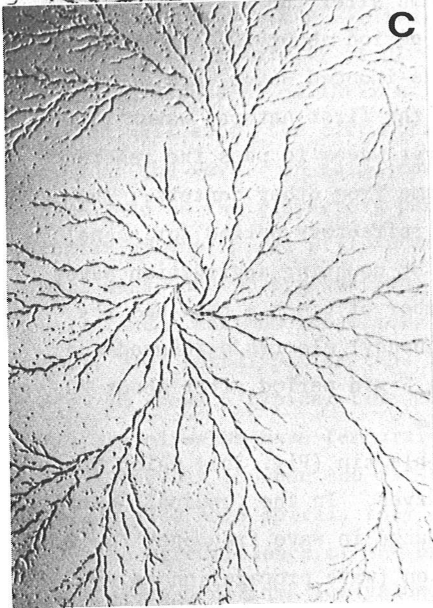
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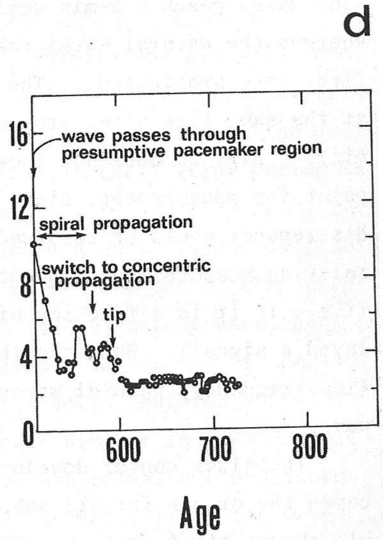
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refractory period. Every nth signal will initiate a new wave if the signal interval is less than the refractory period [nT > Tr > (n - 1)T; T = signal interval, Tr = refractory period].

A plot of wave periodicity against age for a concentric ring aggregation center is shown in Fig. 2b. The figure shows that early waves from the center had a periodicity of 10 min and that after about 3 h (or 17 waves) they underwent a frequency doubling to become periodic at 5 min. This result is typical and it implies that early concentric ring waves are initiated by a periodic signal (with period 5 min) which is gated for the first several waves [6]. The early gating could well be due to the refractory period for signal relaying, since this initially exceeds 5 min but not 10 min and later drops to less than 5 min (see earlier comments).

We can compare the time course for the refractory period, as measured by Robertson [19], using a continuous cAMP source to initiate waves, with the time course of the natural gating. The comparison reveals a discrepancy. The electrode-induced (refractory period) waves reach a 5-min period 120 min after the first wave, whereas the natural waves reach this period about 200 min after the first wave propagated. The electrode-induced waves were started at the same time after starvation as the first natural waves (500 min). Fig. 2b does not show the first waves to pass the reference point for measurement, since these came from other centers. The discrepancy would be resolved if the refractory period for signal relaying measures developmental time by counting aggregation waves (i.e., if it is a function of the number of times a cell has relayed a signal). Both the (high-frequency) electrode-induced and (low-frequency) natural waves reach a 5-min period after about 20 waves.

The older center develops a visible tip (Fig. 2b), which becomes the origin for all subsequent waves. In the (unusual) example shown, tip formation causes no change in wave frequency for 2 h until the center stops wave propagation (wave propagation is replaced by continuous cell movement to the center).

A spiral is a single, continuously propagating wave. It should therefore run indefinitely (and organize an aggregate with the aggre-

gation center at the center of the spiral wave) by signal relaying alone, without any autonomous signaling.

Spirals are initiated when wave fronts are broken appropriately by encountering inhomogeneities in aggregation fields. For example, a wave may encounter an area containing a subcritical density of cells for wave propagation (a hole) [32,37]. The wave will be broken to make two free ends which will circumvent the left and right sides of the hole, respectively, and given no further disturbance, meet to make a single-wave front with a kink distal to the hole. However, if the hole is broken during passage of the wave (e.g., by migration of cells into the hole or by maturation of cells to relaying competence), the outcome can be a number of holes, two of which have one free end of the wave rotating about their circumference and will become the centers of spirals. About 50% of the spirals seen initially have open centers (Fig. 2c) and are probably formed in this way. Spirals can be initiated in other ways, e.g., by interaction of closely successive waves with areas of variable refractory period (Fig. 3) [32,38].

Spiral periodicity is predictable. Newborn spirals will have a period dependent on their origin. In a hole spiral, the initial period will equal the circumference of the hole divided by the velocity of the wave. In solid-centered spirals, the initial period is likely to be the refractory period (Fig. 3). In older spirals, the center of the spiral will invariably become a continuously excitable cell mass (due to aggregation) and the inner free end of the spiral will find a minimum trajectory equal to the refractory wavelength [32]. The spiral period thus always becomes the refractory period. Fig. 2d shows a typical time course for a solid-centered spiral. All spiral waves have temporal frequencies similar to this example. The intervals between the first coils of the spiral are undefined (e.g., in a hole spiral, they depend on the velocity of the wave and the circumference of the hole). The coil period then decreases rapidly to a defined low value (finally one coil per 2.5 to 3 min). This phase is associated with collapse of the open center in open-centered spirals. Spiral propagation eventually ceases and is

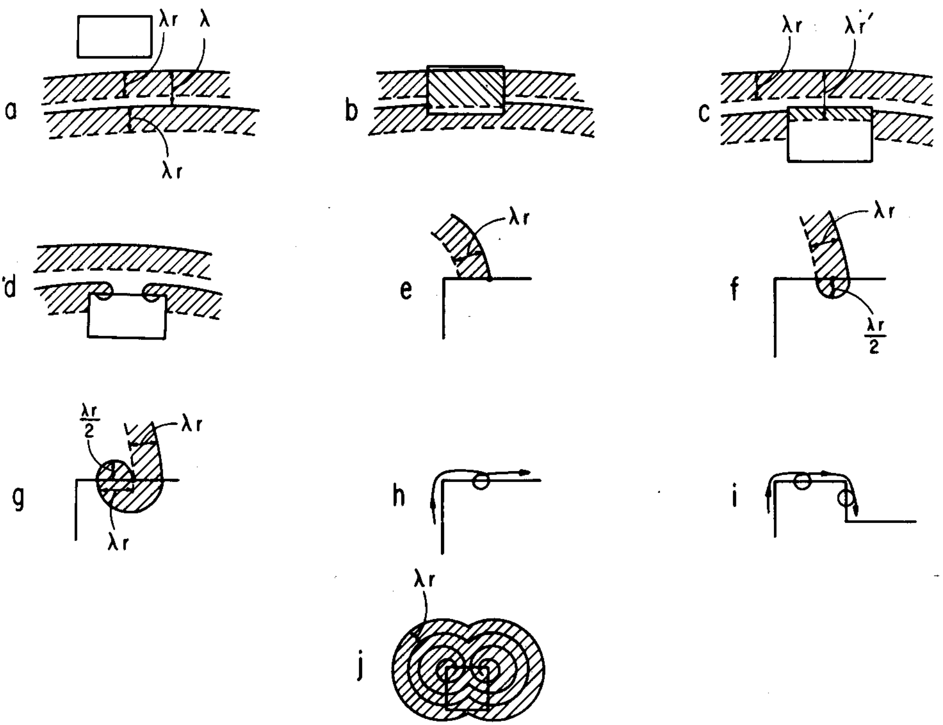

FIG. 3. Initiation of spiral waves via an interaction between two waves and an area of nonuniform refractory period in an aggregation field. (a) Two wave fronts, separated by a wavelength (λ) which slightly exceeds the refractory wavelength of the local aggregation field (λr), approach a patch of tissue with a long refractory wavelength (λr') which exceeds λ. Shaded areas are refractory. Nonshaded areas are sensitive. (b) The second wave front is broken and develops two free ends, one at each side of the patch. (c) The first wave leaves the patch, part of which remains refractory after the refractory zone of the first wave has left the surrounding tissue. (d) The free ends of the second wave propagate around the patch until they reach a part of the patch which has become nonrefractory, and then spiral into it. There is a difficulty in making a stable spiral in this way, and this is as follows: (e) Consider the point in time at which the patch of refractivity becomes sensitive and allows entry of the external wave front. (f) The wave front propagates semicircularly through the patch for a radius of λr/2, until it reaches the back of the refractory zone of the externally propagating wave. (g) It then reenters the external aggregating field and makes a second hemispherical external wave, which cannot reenter the patch as long as the wave trajectory is a straight line because it is perpetually behind the last propagating wave front in the patch and therefore is within the refractory zone (λr') of this wave. (h) The free end of the wave front therefore makes only one turn and generates a transient spiral. (i) A similar process will occur wherever the wave front encounters a convex corner to the patch. (j) Stable spirals may arise if the refractory period of the patch becomes equal to that of the field (λr' = λr). If this occurs in the whole patch before completion of the first turn

generally replaced by concentric ring wave propagation, stabilizing at 2.5 min, which is associated with formation of the tip. The spiral in this example started as a double spiral (two interlocked spiral waves) and was transiently a single spiral before it gave way to concentric propagation. The transient increase in period between the first minimum and the switch to concentric propagation is associated with loss of one of the two spiral waves. Note that the spiral period starts at about 7 min and drops rapidly to 2.5 to 3 min as would be predicted.

In view of their evolution to the minimum period (i.e., the refractory period), one would expect that spirals should be very stable wave forms. They should, within limits imposed by the onset of anisotropy and loss of physical contact between neighboring aggregates, advance the boundaries of their aggregation territories at the expense of neighboring aggregates and eventually take these over, as occurs in other excitable media [26,32]. In fact, one mutant of *D. discoideum* (80) [39] does make stable spirals as the end product of its wave propagation, but in wild-type aggregation, spirals are unstable. Their instability is mysterious, but it is often associated with formation of the tip (and mutant 80 makes no tips).

All late aggregates [spiral (Fig. 2d) and concentric (Fig. 2b)] make an apical tip as previously described. The tip is the origin for any waves made in the late aggregate and these waves are always concentric rings. This is true whether the aggregate originally made spiral or concentric ring waves. Mature tips either initiate high-frequency (one per 2.5 min) concentric ring waves or else initiate continuous cell movement towards the aggregation center. This behavior is mimicked by cAMP microelectrodes set to release cAMP continuously, and I have suggested that the mature tip is a continuous source of cAMP [6,32]. The mature state is usually reached simultaneously with or very soon after appearance of the

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of the spiral, a pair of counterrotating spirals would be expected, as shown in the figure. Other outcomes are possible if only parts of the patch change their refractivity. The periods of refractivity spirals will be equal to the local refractory period of the external medium. This is a time-dependent variable between 2 and 9 min, so their periods will be in this range and may change (decrease) with time.

tip. Occasionally concentric aggregates with tips show 5-min periodic wave propagation for an extended time (>1 h) before mature behavior is manifested. I deduce that some immature tips are either ineffective as pacemakers or periodic with a 5-min period.

E. Mutant Analysis

Most laboratory strains of *D. discoideum* are haploid. It is therefore easy to obtain mutants from them by the same methods as are used to induce mutagenesis in bacteria and yeasts [40]. Some of these mutants have been exploited by using one of two different genetic systems to begin constructing a genetic map of *D. discoideum* and to begin a genetic analysis of aggregation [41-44]. I thought it worthwhile to begin a functional analysis of defects in some morphogenetic mutants by analyzing films of their natural aggregation and of their responses to cAMP pulses delivered from a microelectrode [45,46]. I found that defects occur in each of the competences described above (Table 1). All of the mutants examined grew and moved normally as vegetative cells.

1. Chemotactic mutants were either absolutely unresponsive to cAMP pulses (G50, A5, 7) or made a quantitatively abnormal response (R46) or were apolar (10).
2. Relaying-deficient mutants were tentatively identified as chemotaxis-positive mutants, unable to make waves (or streams) in response to applied cAMP pulses over a range of amplitudes (up to 6×10^{11} molecules). All such mutants examined made some form of organized movement spontaneously, but none made spontaneous waves or streams. Two mutants (ap66, D1) made small, round aggregates (formed by movement of cells directly to the aggregation center), as would be expected if the aggregates are made by a direct chemotactic response to cAMP secreted by centers. One mutant (R46) made mobile bands of high cell density resembling water waves. The bands are often expanding rings and they can be phenocopied by placing wild-type cells on 10^{-3} M cAMP agar.

TABLE 1

Aggregation Competence Defects
in Morphogenetic Mutants of *Dictyostelium discoideum*

Mutant	Source	Defects in aggregation competences	Morphogenetic defects
A5	J. Ashworth	No chemotactic response	No aggregation
7	A. J. Durston	No chemotactic response	No aggregation
50	G. Gerisch	No chemotactic response	No aggregation; final stage: independent amoebas
10	A. J. Durston	Apolar chemotaxis	No aggregation; final stage: independent amoebas
R46	E. Rossomando [47]	Altered time for chemotaxis. Altered chemotactic radius	Final stage: motile bands of amoeba
ap66	G. Gerisch [4,46]	No signal relaying response	Round aggregates; final stage: normal fruiting bodies
1	A. J. Durston	No signal relaying response	Round aggregates; no further morphogenesis
R46	E. Rossomando	No signal relaying response	Final stage: motile bands of amoeba
91	A. J. Durston	Low X3	Many spirals; final stage: slugs
FR17	M. Sussman [48]	Aperiodic autonomous signals from early centers and tips	Final stage: abnormal fruiting bodies

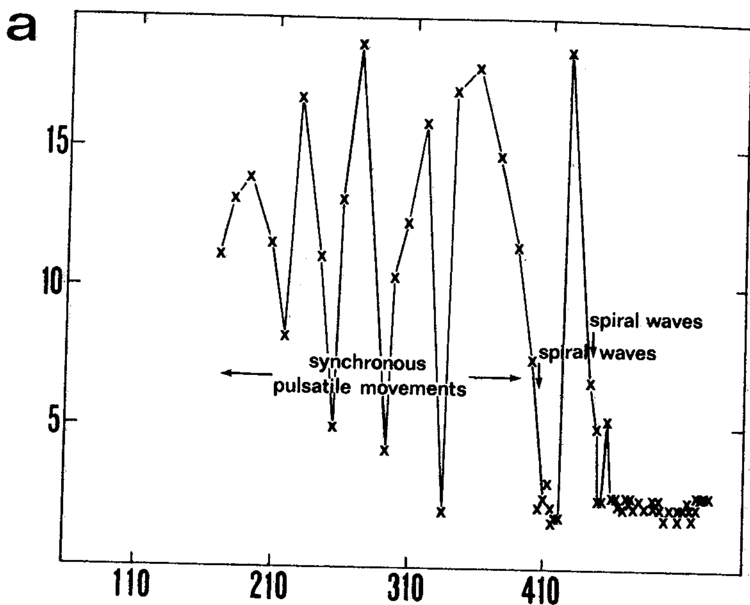
I speculate that, in the wild type, these bands may occur because of cAMP degradation by the slime mold PDE enzymes. Due to PDE, each cell should act as a cAMP sink. Areas of higher cell density should therefore generate depressions in the cAMP landscape, each with a peripheral cAMP gradient. Cells in a high-density area should respond chemotactically to their peripheral gradient to generate a ring of high cell density. The ring will generate a new annular gradient at its outside edge

and will respond to this by continuing to expand.

3. A number of mutants (91, 12, and also Gerisch's mutant 80) make too many spirals (and correspondingly few concentric ring centers). For example, under standard conditions, 91 makes 60% of spirals and wild-type (NC-4) makes 14%. This kind of defect is likely to be due to one of two kinds of problem.
   (a) A low rate of emergence of autonomous cells (low X3) or deficient functioning of autonomous cells, since concentric wave initiation depends directly on autonomous signaling and spiral initiation does not.
   (b) A deficiency in the tip, since demise of spirals is associated with appearance of the tip.

   In fact, the mutant 91A proves to have an X3 which is 20 times lower than that of the wild type [34]. Conversely, Gerisch's mutant 80 makes no tips [39].

4. The mutant FR17 shows defects in early autonomous signaling and in the functioning of its tip. Early FR17 concentric centers initiate aperiodic instead of periodic waves and this phenotype continues after appearance of the tip (which appears at the point of origin of the aperiodic waves). FR17 spiral waves are qualitatively normal (Fig. 4). The concentric ring waves are aperiodic in aggregates with and without tips and are occasionally initiated at points other than the tip in aggregates with tips (this is never seen in wild type). Early FR17 aggregates (synchronous aggregates) make abnormal aperiodic synchronous cell movements which are deduced to be early homologues of concentric ring waves. It is not clear whether the FR17 tip is nonfunctional and merely forms as a morphologically distinct structure in the usual place, or if it indeed makes intermittent signals.



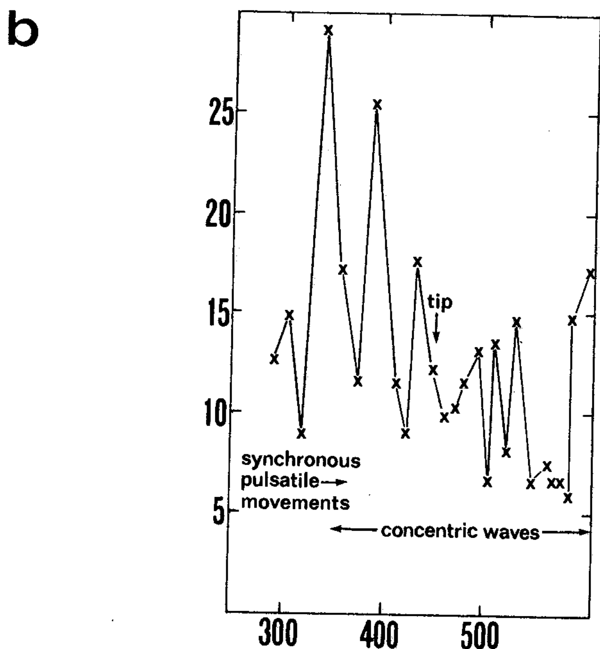


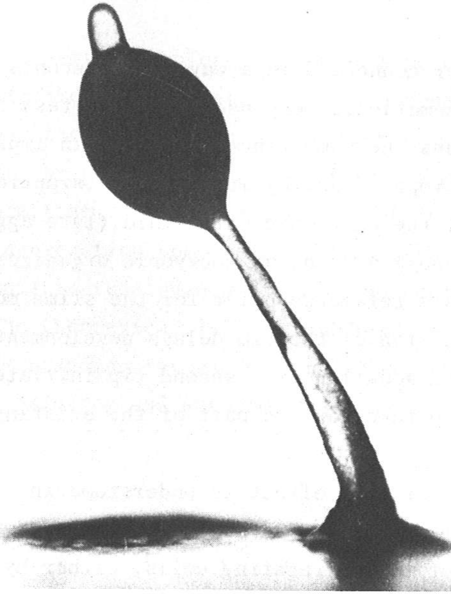
FIG. 4. The mutant FR17 has aperiodic concentric ring waves and normal spiral waves. (a) Shows temporal intervals (ordinate) against developmental age (abscissa) for a synchronous aggregate that develops to a spiral center. (b) Similarly for a synchronous aggregate that develops to a concentric center.

F. Control of Later Development

Three lines of evidence point to involvement of elements of the aggregation control system in later development in *D. discoideum*.

1. We have observed periodic pulsatile movements similar to those seen in aggregation, both in migrating slugs and in erecting fruiting bodies [45,49]. We have analyzed the movement of erecting fruiting bodies [49] and find that these elongate by making precisely periodic movement steps (period 6-½ min) superimposed on continuous movement (Fig. 5). The periodic component of fruiting body movement occurs only during the time when cells are entering the fruiting body stalk (and stops before the end of culmination). The continuous component lasts until the end of culmination and coincides in time with stalk cell vacuolation (which also plays a role in elongating the stalk since vacuolating cells increase their volume) [49,50]. We suspect that fruiting body erection is periodic because of periodic cell movements into the stalk. The periodicity of the fruiting body movements is significantly different from either the mean refractory period or the mean autonomous period during aggregation. Its correspondence with either is uncertain.
2. The polar organization of slugs and of erecting sorocarps can be disrupted by exposure to high cAMP concentrations [51]. The cause of this effect is not known. Cells from mechanically disrupted slugs and sorocarps lack overt chemotactic sensitivity to cAMP for about 50 min after disruption, and then manifest it [50,51]. The cells also reaggregate spontaneously and show normal waves and streams from about 50 min after disruption, suggesting that they have signal relaying and autonomous signaling competences at this time [52].

We must consider the possibilities that slug and sorocarp cells lack the aggregation competences and dedifferentiate to a competent state within 50 min after disruption of the multicellular structure; that they possess these competences but are



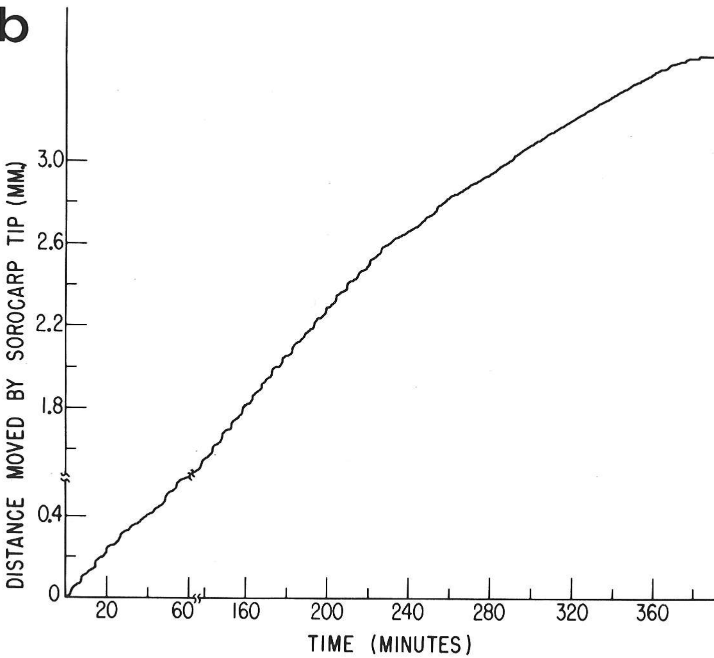


FIG. 5. (a) The later stages of the life cycle of *D. discoideum* show periodic morphogenetic movements. These are most obvious in developing sorocarps at about the stage shown. (b) A distance vs. time plot for elongation of such a sorocarp.

packaged or connected in a way that prevents an immediate, visible chemotactic response under the test conditions; that they possess the competences but make an impaired response due to damage imposed during mechanical disruption.

3. The tip of the embryonic slime mold (late aggregate, slug, and fruiting body) acts as an embryonic organizer [7,8,45]; that is, it acts as a reference point for the slime mold embryonic axis [54]. Excision of the tip delays development until a new tip is made and addition of a second tip initiates a second axis. The new tip takes over a part of the existing structure [7,8, 45].

The basis of this effect is understood in aggregation: it is that the tip is a continuous source of cAMP and therefore controls the movement of the aggregating cells, either by initiating refractory period waves of signal relaying or by inducing continuous centripetal chemotaxis [6,32]. It has now been shown that tips from late aggregates, slugs, and early sorocarps are all equivalent as organizers (i.e., that a tip from any of these stages will organize a new axis in any other stage) [8,45]. It has also been shown that tips from each of these stages continuously secrete cAMP, whereas other parts of slugs and fruiting bodies do not [7]. These results are consonant with independent demonstrations of gradients of bound and secreted cAMP in slugs and fruiting bodies [55,56]. They suggest that the organizing ability of the tip may occur due to its action as a cAMP source.

The results described above justify further enquiry into the roles of chemotaxis, signal relaying, and autonomous signaling in later morphogenesis of *D. discoideum*.

III. FINAL COMMENTS

In the preceding section, I evaluated our understanding, at the cellular level, of the control of aggregation in *Dictyostelium discoideum*. As I hope became clear from the various subsections,

this understanding is very incomplete. We know some of the parameters of the chemotactic, signal relaying, and autonomous competences. We also know the roles of these competences. We know almost nothing about the parameters or importance of cell polarization and contact formation. We should seek to make a more complete description of the aggregation competences so that it will be possible to ask sophisticated and quantitative molecular questions. Experimental [47] and theoretical [57] approaches are already in progress toward understanding the biochemical oscillations which underlie the signal relaying and autonomous periodic competences.

TABLE 2

Some Examples of Periodic Events
in Development of Various Organisms

Organism	Periodic event	Period	Ref.
Acetabularia	Regeneration: contractions of the regenerate and action potentials	minutes	58
Dictyostelium discoideum	Aggregation: cell movement into aggregates	2-10 min	4,6
Dictyostelium discoideum	Culmination: fruiting body elongation	6-½ min	49
Some other cellular slime molds	Aggregation: cell movement into aggregates	minutes	59
Hydra and *Tubularia*	Head regeneration: contractions of the regenerate	minutes	60,61
Campanularia	Stolon growth	10-15 min	62
Locust	Chitin deposition	15-30 min	59
Chick	Waves of optical density, changes in the blastoderm	minutes	63
Chick	Migration of heart mesoderm cells	10 min	59

The real value of the investigations described above is that they make aggregation in *D. discoideum* the only developmental event for which any details of a morphogenetic field are known. We should use the insights obtained with this system to help us understand other morphogenetic fields. There are two approaches:

1. Investigate the role of the aggregation competences in later development of *D. discoideum*. It is especially important to know if they have any relation to the pattern of differentiation. The consensus of the slime mold literature is that this pattern originates as a regulative axial pattern in the slug. Understanding such patterns is perhaps the most difficult problem in pattern formation.
2. Investigate whether morphogenetic fields resembling the aggregation control system in *D. discoideum* are important during embryogenesis in other organisms.

In fact, periodic morphogenetic events and propagating waves of cellular behavior are common during embryogenesis and related phenomena (e.g., regeneration) in organisms as diverse as colonial bacteria, hydroids, and vertebrate embryos. Some examples are listed in Table 2. These periodic events are being investigated in a number of systems [58-63].

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