Dictyostelium discoideum: A Model System for Cell-Cell Interactions in Development

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The cellular slime mold *Dictyostelium discoideum* undergoes a transition from single-celled amoebae to a multicellular organism as a natural part of its life cycle. A method of cell-cell signaling that controls chemotaxis, morphogenesis, and gene expression has developed in this organism, and a detailed understanding of this signaling system provides clues to mechanisms of intercellular communication in the development of metazoans.

THE LIFE CYCLE OF *DICTYOSTELIUM* CONSISTS OF DISTINCT growth and developmental phases. Development is characterized by a stage in which 10^5 single amoebae aggregate to form a multicellular organism (Fig. 1). This spontaneous process is organized by extracellular adenosine 3′,5′-monophosphate (cAMP), which binds to surface receptors that mediate chemotaxis and oscillatory cell-cell signaling. The signaling derives from the capacity of each cell to synthesize and secrete cAMP in response to stimulation by cAMP. Within aggregation centers the concentration of cAMP spontaneously oscillates; each peak initiates a wave of cAMP that propagates through the cell monolayer. The leading edge of each passing wave provides a gradient that briefly orients the chemotactically sensitive cells toward the aggregation center. Cells move up the gradient for several minutes until the peak of the wave reaches the position of the cell. The cells then move in random directions until the next wave elicits another coordinated movement step. About 50 movement steps result in the formation of a multicellular structure (*1–3*). Within the multicellular structure, the signaling system continues to act as a developmental timer and to play a role in morphogenesis and pattern formation. Signaling controls the classes of genes expressed at each stage of development, ensuring that cells differentiate into two types, vacuolated nonviable stalks and durable spores, in a pattern along the axis of the structure (*4–12*).

Biochemistry and Genetics

Free-living *Dictyostelium* amoebae are easily grown and maintained (*13*). The wild-type strains grow to high densities on bacterial lawns with doubling times of 3 hours. About 10^9 cells are typically harvested from a single agar dish (10 cm in diameter). The wild-type strains can also be grown in bacterial suspensions or in axenic media containing particulate nutrients. Axenic strains are available that grow with doubling times of 8 hours in a simple, inexpensive medium of glucose, peptone, and yeast extract. Shaken cultures grow to a density of 10^{10} cells per liter, so that a 100-liter culture yields up to 10^{12} identical cells (*14*). The axenic strains will also grow with doubling times of 24 hours in defined liquid media.

The developmental program is initiated when cells are deprived of nutrients. Environmental parameters that affect the rate of development include cell density and temperature and, when cells are spread on a surface, the wetness of the surface. When these parameters are held constant, the timing of stages of the 24-hour developmental program is reproducible to within 1 hour (*15*).

Growth and development are completely separate, and it is a simple matter to switch between the two modes. When cells are depleted of nutrients, growth ceases and development is initiated. However, differentiation is completely reversible until late stages of the program; on reintroduction of nutrients, cells "erase" their developmental markers and resume growth (*15*). Thus, once developmental mutations are selected, they can be propagated and maintained, and the phenotypes can be expressed again when development is reinitiated. Mutant cell lines can also be stored indefinitely as spores or as frozen amoebae.

The genome of *Dictyostelium* consists of about 40,000 kb, about 1% of the size of the human genome, and is arranged on seven chromosomes (*16*). Although the cells have a true diploid phase, formed from opposite mating types as in yeast, all of the major biological phenomena being studied are expressed when the cells are haploid. Thus, nonlethal recessive mutations affecting processes of interest can be directly scored. It is relatively easy to mutagenize cells, and selection of mutants is limited only by the screening or selection procedure (*17*).

Parasexual diploids form by spontaneous cell fusion at a frequency of 10^{-5} in a population of identical cells. Recessive sensitivity markers can be used to select against the haploid cells and allow survival of diploids (*17*). Thus, if a strain containing a *tsg* marker (cells die at 27°C) is mixed with a strain containing a different *tsg* marker or with a *bsg* (cells cannot feed on *Bacillus subtilis*)-marked strain, then approximately one in 10^5 cells will grow at 27°C or on *B. subtilis* at 27°C, respectively. These diploids can be destabilized, resulting in the production of haploids with a random segregation of the chromosomes. There is a low frequency of mitotic crossing over, which can be used for finer structure mapping (*17*). Mutations can be assigned to complementation and linkage groups (over 100 loci have been mapped) by use of tester strains marked with recessive resistance markers. A series of wild isolates and divergent strains are available for restriction fragment length polymorphism mapping studies, and numerous cloned genes have been assigned to linkage groups (*18*). This information is useful when complementation groups and suspected mutated cloned genes are assigned to the same linkage group.

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Transformation of *Dictyostelium* with exogeneous DNA is a routine procedure (*19–21*). Efficiencies of 10^{-5} to 10^{-3} are readily obtained with either calcium phosphate precipitation or electroporation. Both transient (up to 3 days) and stable cells lines can be constructed. Establishment of a stable cell line requires about 3 hours of culture manipulation followed by approximately 1 week of incubation for the appearance of foci. The available transforming plasmids contain a neomycin transferase gene flanked by an actin promoter and a variety of terminators. Most vectors integrate into the genome in tandem repeats from 10 to 300 copies, although nonintegrating vectors have also been developed by including regions of endogenous plasmids (*22*). Multiple endogenous plasmids occur in *D. discoideum* and other species of slime molds; these plasmids are stably carried at a few to several hundred copies and may facilitate the construction of new vectors (*23*). Vectors have been used for expression of heterologous genes, for analysis of promoter function, and for antisense and homologous recombination experiments.

A number of gene products from other cells have been expressed in *Dictyostelium* with retention of functional properties. Because the cells appear to carry out mammalian-like glycosylation, the system may be suitable for expression and large-scale production of mammalian gene products (*24*); it may be especially useful for receptors and other membrane proteins that are difficult to express in bacteria and may not function in yeast. In addition, developmentally regulated *Dictyostelium* gene products can be expressed during the growth stage of the life cycle when they are normally absent (Table 1).

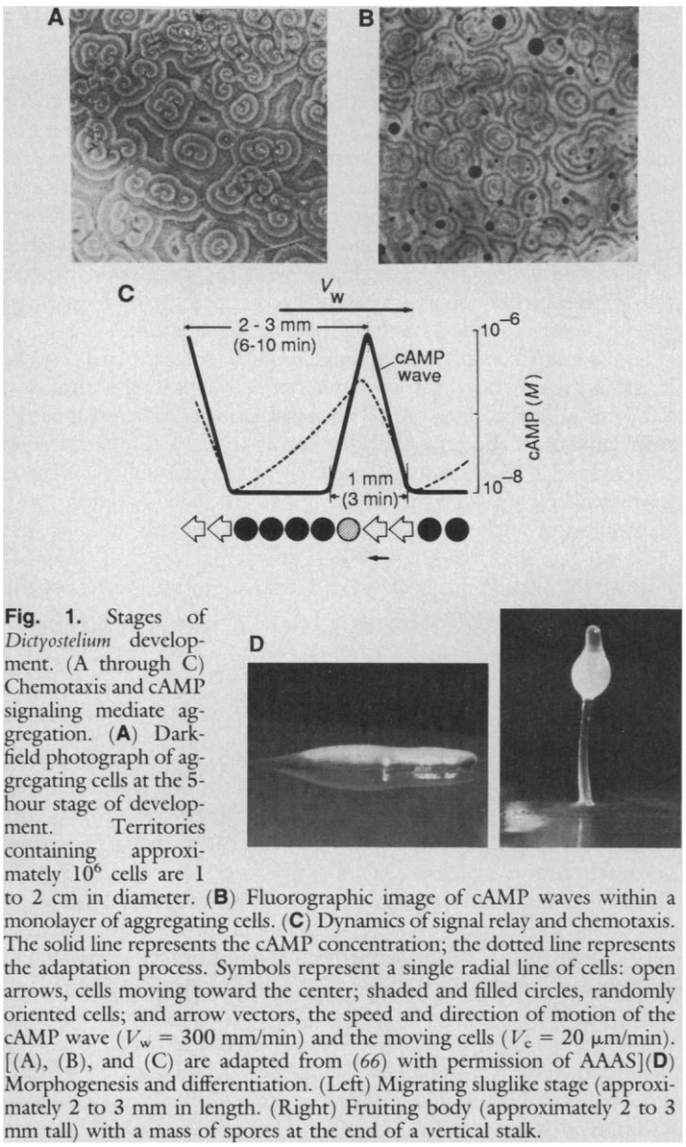

Fig. 1. Stages of *Dictyostelium* development. (A through C) Chemotaxis and cAMP signaling mediate aggregation. (**A**) Dark-field photograph of aggregating cells at the 5-hour stage of development. Territories containing approximately 10^6 cells are 1 to 2 cm in diameter. (**B**) Fluorographic image of cAMP waves within a monolayer of aggregating cells. (**C**) Dynamics of signal relay and chemotaxis. The solid line represents the cAMP concentration; the dotted line represents the adaptation process. Symbols represent a single radial line of cells: open arrows, cells moving toward the center; shaded and filled circles, randomly oriented cells; and arrow vectors, the speed and direction of motion of the cAMP wave (V_w = 300 mm/min) and the moving cells (V_c = 20 μm/min). [(A), (B), and (C) are adapted from (*66*) with permission of AAAS](**D**) Morphogenesis and differentiation. (Left) Migrating sluglike stage (approximately 2 to 3 mm in length. (Right) Fruiting body (approximately 2 to 3 mm tall) with a mass of spores at the end of a vertical stalk.

Table 1. Expression of functional proteins in *Dictyostelium*.

Protein	Source	Developmental stage of expression (hours)	Function assayed	References
Luciferase	Firefly	0, 12, 15	Luminescence	(*60*)
Glucoronidase	Mammalian	12, 15	Fusion protein	(*61*)
$G_\alpha 1$	*Dictyostelium*	0	Multinucleated cells	(*40*)
$G_\alpha 2$	*Dictyostelium*	0	Rescue of *Frigid A*	(*40*)
cAMP receptor	*Dictyostelium*	0	cAMP binding	(*27*)
β-Adrenergic receptor	Hamster	0	Isoproterenol binding	(*62*)
Ras	*Dictyostelium*	0	Multitip phenotype	(*63*)
Ras	Mammalian	0	Multitip phenotype	(*64*)
Phosphodiesterase	*Dictyostelium*	6	Aggregation	(*65*)

The antisense approach can often be applied in *Dictyostelium* to mimic a mutation. Antisense cell lines that express less than 0.5% of the amount of gene product found in the wild type have been constructed for discoidin (an analog of fibronectin), D2 (a serine esterase), myosin heavy chain, and surface cAMP receptors (*25–28*) (Fig. 2). These cell lines display strong phenotypes correlated with the specific loss of the targeted gene product and have helped delineate the roles of these proteins in development. The success of the antisense approach does not appear to depend on the abundance of the targeted protein. Whereas surface cAMP receptors constitute only a small fraction of the cell protein (0.02%), discoidin and myosin heavy chain constitute about 2 and 0.5%, respectively, of cell protein.

In several instances, such as for myosin heavy chain and α-actinin, transformation by homologous recombination with deleted copies of the gene or cDNA has led to integration into and disruption of the endogenous gene (*29–31*). In the case of myosin, cells were transformed with a construct containing the 5′ end of the gene with a nonfunctional promoter. A single crossover occurred and the cells began to express the NH_2-terminal half of myosin, heavy meromyosin. This construct was excised spontaneously with reversal of the phenotype when cells were removed from selection (*29*) (Fig. 2). In the case of α-actinin, cells were transformed with an internal fragment of the coding sequence. Again a single crossover occurred, resulting in two nonfunctional copies of the gene, and the gene product was either not synthesized or was lost by degradation (*30*). The studies of myosin heavy chain have been extended by transforming cells with regions flanking the coding sequence separated by the neomycin transferase gene. In several instances, this type of construct resulted in a double crossover and irreversible disruption of the gene (*31*).

These and other recent observations contain the seeds for growth of a powerful genetic system. It is possible to complement "null" mutants with cloned genes so that the wild-type phenotype is restored (*32*). Moreover, a thymidine synthetase mutant has been complemented with a clone from a genomic library (*33*). Mutant complementation with genomic libraries will probably become a more widely used technique as improvements in transformation efficiency are achieved. It should be possible to introduce mutations into endogenous genes and to clone new genes by complementation of mutants. *Dictyostelium* is especially well suited for studies of signal transduction, motility and chemotaxis, cell-cell communication, gene expression, and pattern formation, and because of the similar-

ity of many functions and genes between *Dictyostelium* and mammalian cells, these studies may provide general insights.

Signal Transduction

Transmembrane signaling systems in *Dictyostelium* seem to be essentially the same as those in mammalian cells (Fig. 3). Surface cAMP receptors have many of the features of receptors that are linked to G proteins. Like bovine rhodopsin and the β-adrenergic receptor, the cAMP receptor has seven putative membrane-spanning domains and a serine- and threonine-rich COOH-terminus (*27*). The affinity of receptors for cAMP is reduced by guanine nucleotides, and a primary effector, adenylate cyclase, is stimulated by guanosine triphosphate in vitro (*34–36*).

These guanine nucleotide–dependent activities can be independently affected by mutation. A series of mutants, designated *Frigid*, that fail to enter the developmental program even when provided with exogenous cAMP has been isolated (*37*). In several mutants, belonging to the complementation group *Frigid A*, guanine nucleotides do not influence cAMP binding. A second series of mutants, designated *Synag*, has also been characterized. In these mutants, adenylate cyclase and the cAMP signaling response are no longer sensitive to guanine nucleotides, although repeated stimulation with cAMP still is able to induce chemotaxis and gene expression (*38*). Cells contain two homologs of the α-subunits of mammalian G proteins that are 45% identical and a β-subunit that is 63% identical to their mammalian counterparts at the amino acid level (*39*). *Frigid A* cells carry deletions or mutations in one of the two G protein α-subunits, $G_{\alpha}2$ (*40*). A defect in a *Synag* mutant has not been associated with a mutation in a G protein subunit. Homologs of G protein α and β subunits have also been found in yeast, where they participate in the α/a-mating factor response (*41*). Thus G protein–linked transmembrane signaling systems appeared early in the evolution of eukaryotic cells.

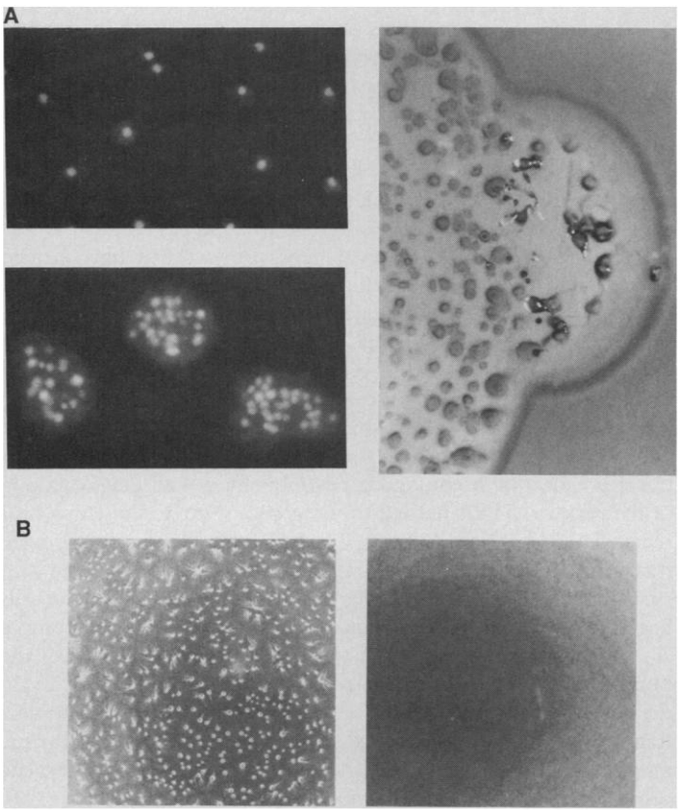

Fig. 2. Mutations with phenotype. (**A**) Disruption of myosin heavy chain by homologous recombination. (Left) Multinucleate morphology of cells expressing the NH_2-terminal portion of myosin. Control (upper) or mutated (lower) cells were allowed to attach to slides, then fixed and stained with 4′,6-diamidino-2-phenylindole. Individual nuclei are 3 μm in diameter. (Right) Reversion of the phenotype. Transformed cells were grown on a bacterial lawn in the absence of drug selection. The left portion shows a region where the cells have depleted the bacteria and formed mounds that did not develop further. A reversion event, recorded in the center of the photograph, is visible as an outgrowth (1 cm across) from the leading edge of the feeding fruit. The reversion gave rise to cells that grew faster, formed normal fruiting bodies, and contained intact myosin heavy chain. [(A) is adapted from (*29*) with permission of AAAS] (**B**) Antisense transformation mimics receptor mutation. Vectors were designed to express full-length antisense RNA of the cAMP receptor in growing cells. The control transformants (no receptor sequence in vector) (left) differentiated as wild-type cells, aggregating with streams of cells entering the centers. Antisense transformants (right) remained as a uniform cell monolayer, shown here at the 7.5-hour stage of development. A 5 by 5 cm section of each monolayer is shown. [(B) is adapted from (*27*) with permission of AAAS]

The cAMP receptors undergo a process of desensitization similar to that observed for related mammalian receptors. Desensitization involves both down-regulation and adaptation (*42*), and the adaptation properties of several receptor-mediated cellular responses, such as guanosine 3′,5′-monophosphate (cGMP) accumulation, myosin phosphorylation, and adenylate cyclase activation, have been studied. Cells respond to increases in the fractional occupancy of surface receptors, and when occupancy is held constant, responses subside within a few minutes. The magnitude of a response is proportional to the fractional increase in receptor occupancy, and this is true for the initial challenge with cAMP or for any subsequent increment in the amount of cAMP. When the stimulus is withdrawn, adaptation decays and cells regain sensitivity in about 15 min. Adaptation is probably due to a rapid, extensive ligand-induced phosphorylation of the receptors. The kinetics and ligand-concentration dependence of phosphorylation correlate closely with the adaptation process (*43*). This reversible phosphorylation cycle appears to be an essential feature of the biological oscillator during aggregation.

Signal transduction through surface cAMP receptors participates in a multitude of developmental functions. It appears that there is a family of related surface cAMP receptor subtypes. The existence of such families of receptor subtypes seems to be a common theme within the class of G protein–linked receptors. Each subtype may be responsible for one or a few of the cAMP-mediated functions (*44*). Expression of antisense RNA of the cAMP receptor subtype first characterized may block expression of all the receptor subtypes because the antisense cell lines are defective in most aspects of the developmental program (Fig. 2).

Motility and Chemotaxis

The movement and chemotactic responses of *Dictyostelium* cells closely resemble those of amoeboid-like cells in higher eukaryotes. The cascade of physiological and biochemical responses that occurs in response to cAMP in *Dictyostelium* is similar to that triggered by chemoattractants in leukocytes and macrophages (*45*). In the absence of stimuli, *Dictyostelium* cells move at between 7 to 10 μm/min and extend pseudopods every 30 s at a rate of about 30 μm/min. The cells contain many of the contractile and cytoskeletal proteins found in higher eukaryotes, including actin, myosin, gelsolin, tubulin, and α-actinin. Actin seems to play a central role in motility and chemotaxis. Filamentous actin (F-actin) is present in the advancing pseudopods and forms a meshwork at the sides and posterior of the cell; its extent of polymerization is transiently increased upon addition of chemotactic stimuli (*46*). Myosin heavy chain is found in

punctate regions of the cytoplasm and is concentrated in the posterior ectoplasm (*47*); it redistributes to the cell periphery in activated cells.

A large number of mutants defective in chemotaxis have been isolated, and although only a few have been characterized, they have helped in defining which components are essential for motility and chemotaxis. For example, *Frigid A* mutants are unable to undergo chemotaxis, suggesting that a G protein is essential for this process (*48*). Although in *Synag 7* mutants the activation of adenylate cyclase is defective, their chemotactic response is normal, suggesting that intracellular cAMP does not participate in this process. *Streamer F* mutants lack cGMP-specific phosphodiesterase, so that there is a persistent elevation of intracellular cGMP (*49*). As a result, the cells remain elongated for several minutes after removal of the external gradient of chemoattractant (*50*). The heavy chain of conventional myosin, α-actinin, and gelsolin (which have all been disrupted) do not appear to be essential for motility and chemotaxis. Cells also contain shorter forms of myosin heavy chains, which may be involved in motility (*51*). Conventional myosin appears to play a role in cytokinesis, as cells lacking the heavy chain become multinucleate (*29*) (Fig. 2).

Cell-Cell Communication

The cell-cell signaling system in *Dictyostelium* appears to be unlike that of any other member of the biological kingdom. It is a simple method of communication within a population of identical cells, all of which secrete and respond to the same molecule. Similar communication systems may be used in other cell types, however, possibly during embryogenesis or regeneration when groups of identical cells must spontaneously organize.

The cAMP signaling system does not supplant other mechanisms of cell-cell interaction. Since all species of slime mold appear to use cAMP signaling in later development but do not form interspecies structures, there must be additional modes of cell-cell recognition. Specific cell adhesion molecules (CAMs) found in *Dictyostelium* may serve this function (*52*). The best characterized CAM is the protein referred to as GP80 or "contact sites A," which shares regions that are homologous to CAMs from higher eukaryotic cells, such as L-CAM and N-CAM in birds and mammals (*53*). *Dictyostelium* also contains an analog of fibronectin, discoidin, which mediates cell adherence to the substrate. This protein contains the RGDX (Arg-Gly-Asp-X) sequence commonly found in adhesion proteins (*54*).

Gene Expression

Most of the known components of the sensory system, such as surface cAMP receptors, adenylate cyclase, and the G protein subunits, are subject to developmental regulation. The periodic cAMP signaling, in turn, regulates the expression of these components as well as that of other developmentally controlled gene products. Receptor-mediated events also appear to regulate late cell–type specific differentiation, pattern formation, and morphogenesis. Characterization of mutants such as *Frigid A* and the *Synags* indicate that signal transduction pathways that include G proteins regulate these events (Fig. 3). The effectors of these G proteins are unknown, although they may include phospholipase C.

Cells must be exposed to cAMP in a specific regimen to induce expression of each of the gene classes. Low, constant concentrations of exogenous cAMP inhibit the early developmental program, whereas repeated application of cAMP at 6-min intervals accelerates the process. In contrast, late cell type–specific gene expression

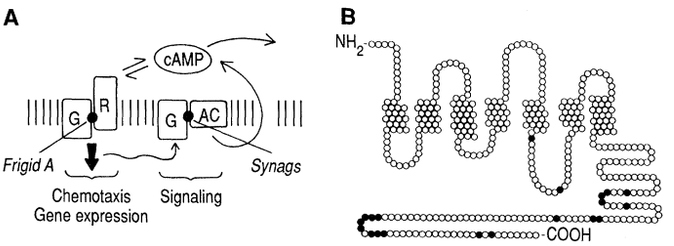

Fig. 3. Transmembrane signaling system. (**A**) Diagram of cAMP signaling system, including interactions among receptor (R), G proteins (G), and adenylate cyclase (AC). (**B**) Model of the cAMP receptor. The seven hydrophobic domains are arranged as α-helices in the lipid bilayer. The proposed extracellular domains are shown above the α-helices, and the intracellular domains, including the serine-rich COOH-terminus (serines are filled), are shown below these transmembrane helices. [(B) is adapted from (*27*) with permission of AAAS]

depends on continuous application of cAMP. These observations suggest that the receptor-mediated responses that activate early gene expression adapt, whereas those that activate the late cell–type specific genes do not. Thus, the cAMP oscillator, regulated by receptor-mediated phosphorylation (and dephosphorylation), may be an essential part of the developmental timer.

Morphogenesis and Pattern Formation

As cells that are initially identical aggregate to form the multicellular "slug" structure, a simple pattern appears along its axis (Fig. 1). A sharp demarcation separates cells within the posterior three-fourths of the slug, which express "prespore" markers, from those at the anterior, which do not express these markers. The anterior cells later differentiate into stalk cells and are designated "prestalk" cells. A variety of proportioning mutants that affect the ratio of cell types have been isolated.

This morphological pattern seems to be achieved through the establishment of a pattern by chemotactic cell sorting and through subsequent position-dependent differentiation. Cells that have undergone periods of starvation, that enter the developmental program from early stages of the cell cycle, or that have been grown on glucose-deficient media preferentially sort to the anterior region. Subsequent prespore marker accumulation is confined to the posterior region of the slug, and later, stalk cell differentiation takes place at the tip and ventral side of the structure, indicating that there are position-dependent developmental cues (*3*).

The tip of the slug displays properties of an embryonic "organizer." If a slug is severed just behind the tip, the tissue connected to the tip continues to migrate. However, migration of the remainder ceases, the cell mass re-forms a mound, and a new tip appears before morphogenesis continues. Excised tips, grafted to host slugs, define new axes and thereby cause several smaller slugs to form and separate. These organizational properties of the tip appear to derive from its capacity to generate oscillatory cAMP signaling (*55, 56*).

In addition to cAMP, other compounds such as adenosine, differentiation-inducing factor (DIF), and ammonia have been implicated as morphogens in the developing system (*57–59*). Adenosine appears to antagonize the actions of extracellular cAMP, and its production in anterior regions has been postulated to inhibit the formation of additional tips and to prevent anterior cells from expressing prespore genes. DIF has been identified as 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-1-hexanone, a lipid-like compound that is essential for stalk cell differentiation. Certain marker mRNAs have been shown to be dependent on DIF for expression and to be expressed only in anterior cells. In this sense

DIF displays the properties of a morphogen, a compound described in theories of embryonic pattern formation as being able to induce cell differentiation in a region of a responsive field of cells. Ammonia appears to promote spore cell formation by counteracting the effects of DIF.

Conclusions

The mechanisms of signal transduction in eukaryotic microorganisms appear to be similar to those in mammalian cells, and thus genetics can be applied to study relevant signal transduction phenomena. The role of signal transduction in processes such as motility and chemotaxis are most easily addressed in *Dictyostelium*. The relation of the signaling properties of individual cells to the behavior of aggregates of cells is more clearly understood in *Dictyostelium* than in other organisms. However, there are major apparent differences between *Dictyostelium* development and that of embryos. In *Dictyostelium*, a cell mass forms from the aggregation of a population of independent cells; in an embryo, a highly ordered cell mass forms from the division of a single cell. Whether the rules for the steps of morphogenesis and pattern formation that follow, and appear similar in each case, are more fundamental than these differences will determine the future utility of *Dictyostelium* as a model for development.

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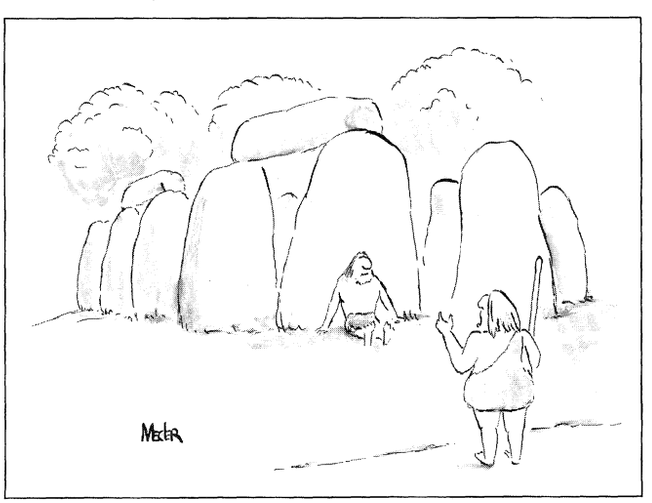

" Say, Og, you got the time?"