Propagation of traveling waves in excitable media

Brian M. Sager
Rowland Institute for Science, Cambridge, Massachusetts 02142 USA, and Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138 USA

Traveling waves transmit information through space. Wave patterns arise in phenomena as diverse as the migrating action potentials of neurons [Hodgkin and Huxley 1952], spiraling bands of electrical excitation detected in cardiac muscle [Davidenko et al. 1992], and the movement of calcium within differentiating Xenopus laevis oocytes [Lechleiter et al. 1991]. In each of these cases, an excitable medium serves to promote wave propagation. An excitable medium is typically comprised of a continuous set of locally excitable regions, which can be both independently stimulated and inhibited. These media exhibit a sensitivity threshold below which the media persist undisturbed at a stable resting state. While subthreshold perturbations are rapidly diminished, greater-than-threshold signals induce an abrupt local transformation within a portion of the medium. Shortly after this change occurs, the region becomes transiently refractory to further perturbation, after which it relaxes to the resting state [Tyson and Keener 1988].

If a localized medium-transforming signal can bring neighboring regions to threshold before being damped out, a signal wave can spread. The kinetic link between the propagation of a transforming signal and the control of its refractory state underlies the mechanism of movement of many types of traveling waves, including those of a Belousov-Zhabotinsky [BZ] reaction, the oxidation and decarboxylization of malonic acid by bromate, and the developmental waves of Myxococcus xanthus and Dictyostelium discoideum cells. In the multicellular prokaryote M. xanthus, traveling waves result from cell-contact-mediated intercellular C-signaling, leading to the movement of cell-cell signaling waves in the absence of bulk cell movement. The C-factor, which is cell bound, acts over a very short range. In contrast, D. discoiudium ameboid cell migrations are directed in part by diffusing cAMP, which is alternately perceived and produced by relays of aggregating cells. Both chemical autocatalysis and positive autoregulation can direct the formation of waves in excitable media. This review compares these morphogenetic mechanisms and reveals their common requirement of a coordinated pair of propagator and controller species, which regulate the excitation and recovery of the medium, respectively.

Morphogenesis in chemical systems

Traveling chemical waves delineate the movement of autocatalytic reaction fronts. In chemical systems, wave formation and migration can occur as a result of the interplay between diffusion and the coupled production or destruction of an ionic species. A wave is generated by local perturbation of a medium, that is, by an increase in the concentration of a substance above a threshold value required for autocatalytic production. This is typically accomplished by induction of a spatial variance in the distribution of that species. Perturbation can be achieved by several mechanisms, including the application of a low-voltage pulse of electricity to the medium [Mori et al. 1991], immersion of silver electrodes in the reaction vessel [Foerster et al. 1988], or by addition of a chemical species to the chemical chamber [Jorne 1980].

Once initiated, autocatalysis further increases the local concentration of the perturbing species within that reaction space. This increase establishes a concentration gradient of the species and results in the diffusion-driven transport of the substance into neighboring domains. Upon experiencing a concentration above threshold, the neighboring regions propagate both the autocatalytic production and the subsequent transport of the substance. What level of chemical complexity is required for such a reaction system to self-organize wave patterns?

The BZ family of reactions represent a classical oscillating reaction system [Belousov 1958; Zhabotinskii 1964; Field and Noyes 1974; Jorne 1980; Mori et al. 1991]. One particularly well-characterized reaction in the BZ family is the oxidation and decarboxylization of malonic acid by bromate in the presence of a catalyst such as the redox couple and indicator ferroin [the tris (1,10-phenanthroline) ferrous sulfate complex]. The initial system can contain a mixture of sodium bromide, sodium bromate, and malonic acid [Müller and Plessser 1995]. The reaction has been approximated by Ross et al. [1988] as:
In this system, two coupled pathways drive the bromination of malonic acid. A reducing pathway consumes bromide and generates a low basal concentration of HBrO₂, while an autocatalytic oxidizing pathway produces a high HBrO₂ concentration. If the HBrO₂ concentration is low, the reducing pathway is preferred, and the HBrO₂ concentration locally rises. If this increase becomes greater than the threshold concentration required for initiation of the oxidizing pathway, then the oxidizing pathway undergoes a self-amplification, and the HBrO₂ concentration continues to build. Ultimately, however, the oxidizing pathway will fail to produce further HBrO₂, as a result of the conversion of ferroin to ferrin. Ferrin quickly reacts with bromomalonic acid to produce bromide ion. Over time, the ferrin regenerates, and the reaction can proceed. Thus the local consumption and regeneration of the ferroin sets the extent of recovery of the medium’s excitability.

Wave propagation can proceed by one of two routes. First, diffusion of the HBrO₂ from an excited region of the reaction medium to neighboring regions will trigger the autocatalytic oxidizing pathway, exciting these local domains and resulting in the formation of moving bands. Second, a local depletion of bromide will arrest the reducing pathway, driving an increase in the HBrO₂ concentration above the threshold required to initiate the autocatalytic oxidizing pathway [S. Müller, pers. comm.]. In either case, as the band propagates, the medium in which it travels is transiently converted from a reduced to an oxidized state, which can be detected by a color change as ferroin, normally red in solution, converts to blue-colored ferrin [Figs. 1B and 2]. Many robust spatial patterns can form as the BZ reaction proceeds. Spiral-shaped and rotating pinwheel waves have been reported [Noszticzius et al. 1987], as have three-dimensional scroll waves, where a scroll band of oxidation rotates in solution [Kenner and Tyson 1988].

The abrupt autocatalytic production of bromous acid provides the excitatory input for the BZ reaction and has therefore been identified as the propagator species, as it propagates the excitation wave [Fife 1984]. In this reaction the propagator species is transported by bulk diffusion. The rate and direction of wave propagation is controlled by the concentration of ferroin, whose interaction with bromous acid governs the recovery of the system from its excited state. For this reason ferroin has been termed the controller species (Table 1; Fife 1984). The wave period is set by the balance between the rate of autocatalysis and the slow consumption of the autocatalytic inhibitor.

What properties do oscillating chemical reactions share with biological wave-forming systems? Whether chemical or biological, excitable media can be identified and compared by determining the propagator and controller species responsible for wave movement. Furthermore, the mode of transport of each species constrains the mechanism of wave propagation in both space and time. In biological systems, medium-perturbing signals may be encoded by the concentration of a migrating reaction front containing a chemical species, generated by changes in the concentration of a protein or low-molecular weight substance, or induced by the properties or locations of cells in a developing tissue. Morphogenetic signals can be cell bound, as is C-factor during Myxococcus development, or diffusible, as are the cAMP waves driving Dictyostelium differentiation. What are the propagator and controller species in these developmental systems?

**Morphogenesis of Myxococcus xanthus**

In the developmental life cycle of the soil-dwelling bacterium *Myxococcus xanthus*, multicellularity has evolved as a strategy for survival [Dworkin 1996]. *M. xanthus* cells are shaped as rods 5 µm long and 0.5 µm wide [Kaiser 1979]. These gram-negative bacterial cells move and feed cooperatively when nutrients are plentiful, migrating en masse and pooling their secreted bacteriolytic, proteolytic and hydrolytic enzymes to trap microbial prey [Burnham et al. 1984; Rosenberg et al. 1977]. Upon amino acid starvation, cells self-organize into dense, reg-

**Figure 1.** Traveling waves in biological and chemical systems. **(A)** Ripples in wild-type *Myxococcus xanthus*. Representative morphology of rippling waves in submerged culture. Dark bands show regions of high cell density. Bar, 100 µm. **(B)** Spiralizing BZ waves in the ferroin-catalyzed BZ reaction (photograph courtesy of Stefan Müller). Initial concentrations: 48 mM NaBr, 342 mM NaBrO₃, 95 mM malonic acid, 376 mM sulfuric acid, 3.5 mM ferroin. Light bands indicate regions of oxidation. Bar, 1000 µm. **(C)** Wave movements in *Dictyostelium discoideum* (photograph courtesy of Peter Newell). Dark bands depict regions of inwardly aggregating cells. Bar, 1000 µm.
Propagation of traveling waves

An [HBrO₂]-dependent oxidizing pathway drives the autocatalytic production of HBrO₂, whereas a reducing pathway generates HBrO₂ and consumes bromide. MA, malonic acid; BrMA, bromomalonic acid. (Adapted from a drawing kindly provided by S. Müller, Max Planck Institut für Molekulare Physiologie, Dortmund, Germany.)

Figure 2. Two coupled pathways drive HBrO₂ formation in the BZ Reaction. An [HBrO₂]-dependent oxidizing pathway drives the autocatalytic production of HBrO₂, whereas a reducing pathway generates HBrO₂ and consumes bromide. MA, malonic acid; BrMA, bromomalonic acid. (Adapted from a drawing kindly provided by S. Müller, Max Planck Institut für Molekulare Physiologie, Dortmund, Germany.)

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Table 1. Propagation and control of wave-forming excitable media

<table>
<thead>
<tr>
<th>Reaction system</th>
<th>Propagator species</th>
<th>Controller species</th>
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<tr>
<td>Belousov-Zhabotinsky</td>
<td>bromous acid</td>
<td>ferroin</td>
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<td><strong>Myxococcus xanthus</strong></td>
<td>C-factor</td>
<td>buildup of ferroin</td>
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<td><strong>Dictyostelium discoideum</strong></td>
<td>cAMP</td>
<td>heterogeneous cell</td>
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Intercellular signaling

Intercellular signaling coordinates rippling and fruiting body formation (Kuspa et al. 1986; Kroos and Kaiser 1987; Kim and Kaiser 1990a). Extracellular complementation screens have revealed five classes of intercellular signaling mutants (Hagen et al. 1978; LaRossa et al. 1983; Downard et al. 1993). When developed alone, mutant cells of a given signaling class cannot undergo morphogenesis. When mixed in a 1:1 ratio with cells from a different signaling class, or with wild-type cells, however, development proceeds normally. These experiments argue that cells deficient in different signaling pathways can cross-rescue each other when mixed. Developmental rescue of otherwise incompetent signaling mutants by wild-type cell extract has been used as a bioassay in the purification of solubilized C-factor, which was identified as a homodimeric, 24.5-kD membrane-associated protein encoded by the csgA gene (Kim and Kaiser 1990a, b; Hagen and Shimkets 1990; Lee et al. 1995). The csgA gene shares sequence homology with an ancient family of short-chain alcohol dehydrogenases (Baker 1994), and may function as a cell-surface associated NAD(P)+ coenzyme-binding protein (Lee et al. 1995). Although an enzymatic activity for csgA has not yet been described, recent work supports such a hypothesis. In particular, Lee and Shimkets (1996) have demonstrated that it is possible to suppress a strain exhibiting defective C-signaling by introducing second-site mutations in the extragenic socABC operon. When mixed 1:1 with csgA mutant strains, these suppressor strains can efficiently rescue the C-signaling mutants, arguing against csgA as the signal molecule per se, and for a restoration of C-signal-like activity via a C-signal independent enzymatic pathway.
Regulation of gene expression and cell differentiation

What is the role of C-signaling in the developmental program? The C-signal is required for at least three morphologically distinct functions: Aggregation, fruiting body formation, and the transformation of a rod cell into a myxospore all require C-factor activity [Kim et al. 1992]. In addition to permitting progression through these morphological landmarks, C-signaling has been shown to govern the proper expression of a set of developmentally induced genes. To monitor the progress of differentiating *Myxococcus* cells, transcriptional lacZ fusions were made to several developmentally regulated promoters. Several promoter fusions were identified that displayed temporally distinct expression patterns in developmentally wild-type cells [Kroos et al. 1986]. The dependence of these fusions on the developmental signals described above was examined by transducing each fusion into strains deficient in a particular class of cell–cell signaling and comparing their expression patterns to wild type.

These experiments revealed a hierarchically regulated cascade of genes functioning during development [Kroos and Kaiser 1987]. The set of gene fusions dependent on C-factor activity were shown to be expressed relatively late in development: The onset of expression of the earliest promoter fusion occurs 6 hr after starvation, whereas the latest occurs two days into the developmental program [Kroos and Kaiser 1987]. Several late-expressing lacZ fusions were isolated that exhibited higher levels of β-galactosidase activity in spores than in developing rods [Kuspa et al. 1986]. These spore-specific promoter fusions were found to require C-signaling in order to achieve proper expression levels [Kroos and Kaiser 1987]. Thus C-signaling simultaneously regulates a multiplicity of intracellular and intercellular developmental circuits and therefore serves as a central control point in the development of *Myxococcus*.

A clue to one intercellular function of C-factor is revealed by the developmental phenotypes of mutants defective in C-factor production. In addition to their inability to aggregate into fruiting bodies, these mutants exhibit a failure to ripple [Shimkets and Kaiser 1982] This incompetence suggests that intercellular C-signaling may coordinate developmental cell movement. How might C-signaling direct the movements of rippling cells?

Intracellular transduction

*M. xanthus* cells move by gliding on a solid substrate and direct their migration patterns by altering the rate at which they reverse direction. The frequency of cell reversal is known to be under the control of the frz genes [Blackhart and Zusman 1985]. Members of this set of genes share homology with the two-component response regulator pathways found in enteric bacteria (McBride et al. 1989; Stock et al. 1989). In particular, the cell-reversal-inducing signal transduction pathway requires the proper activity of FrzCD, which is both structurally and functionally similar to the cytoplasmic domain of a methyl-accepting chemotaxis protein. FrzCD can be methylated at five distinct glutamate residues. Levels of FrzCD protein methylation are controlled by the activities of FrzF, a methyl-transferase, and FrzG, a methyl-esterase [McCleary et al. 1990, McBride et al. 1992]. Regulation of the FrzCD methylation level alters the signaling sensitivity of the protein, presumably allowing it to respond to a wide dynamic range. Signal transmission proceeds as FrzCD modulates the phosphorylation level of the FrzE kinase. Within FrzE, intramolecular phosphate is transferred from one region of the protein to another [McCleary and Zusman 1990], upon which the autophosphorylated FrzE kinase signals the gliding motor to reverse. The molecular nature of the motor and the mechanism underlying motor reversal are not known.

A growing body of evidence argues that C-signaling is transduced by the Frz transduction system and serves to coordinate cell reversals. Purified solubilized C-factor modulates the reversal rate of single cells [Sager and Kaiser 1994]. This modulation is likely to be controlled by the frz system, as recent experiments have shown that C-factor can alter the methylation state of the frzCD protein in vitro [Søgaard-Andersen and Kaiser 1996]. Further evidence for C-signal transduction by the Frz system comes from a screen for potential C-factor receptor mutants. Several transposon-insertion mutants have been isolated that fail to respond to C-factor, although they can produce C-factor at wild-type levels. These mutations, which arrest at the same developmental stage as a csgA null mutant, have been genetically and physically mapped to the Frz locus [Søgaard-Andersen et al. 1996].

Cell reversals, presumably under the control of the frz system, are required for proper rippling. Frz mutants unable to regulate their cell movements cannot ripple. Moreover, videomicroscopic analyses of single labeled cells have revealed periodic cell reversals within ripples. Individual cells typically travel a distance on the order of a wavelength and then reverse [Sager 1994]. Taken together, these lines of evidence suggest that C-signaling is transduced by the Frz system, which in turn regulates the rate of cell reversal within ripples (Fig. 3). As C-factor is membrane-associated, how might C-signaling coordinate intercellular communication during rippling?

Cell movements within waves

Cell motility is required for C-signal transmission. Although non-motile cells can produce and respond to C-factor, they can neither express C-factor-dependent genes nor sporulate because they cannot transmit C-factor [Kim and Kaiser 1990c]. Signal transmission can be restored by reconstruction in non-motile cell cultures of both the end-to-end and side-by-side alignment normally found in cultures of motile cells. Alignment of arrays of non-motile cells along their long axes permits these cells to achieve the high density characteristic of motile aggregates. Under these conditions C-signal-dependent gene expression is restored to near wild-type levels [Kim and Kaiser 1990d]. The epigenetic rescue of C-signal-dependent gene expression in non-motile cells by forced
Cyclic movements of cells occur within a high-density culture, suggesting that cells are unlikely to retrace their steps in an absolutely periodic fashion. Rather, the movement of a given cell might typically follow a similar but not identical path as it cycles within a ripple. An aperiodicity in a recurrent process is a potential indicator of chaotic behavior in that system (Field and Györgi 1993). Thus the paths of single cells could be chaotically cycling within ripples. Indeed, chaotic cellular behavior may promote pattern formation. Contact-mediated C-signal transmission chemically couples neighboring cells with one another, and coupled chaotic oscillators can produce spatial patterns. In computer-modeled simulations of morphogenetic fields, arrays of coupled oscillators have been shown to drive the formation of spiraling concentric bands and periodic wave patterns reminiscent of rippling waves (Klevescz et al. 1991).

**C-signaling and directed cell movement**

The rippling wave model described above suggests that the head-to-head cell contacts brought about by the collisions of countermigrating ripples may trigger cell reversals. It is likely, however, that different patterns of motility result from the various densities and geometric arrangements found within cell groups. In rippling waves, cells are frequently arranged in wide linear bands, side-by-side, with occasional head-to-head contacts occurring mainly during ripple collisions. Under these conditions, cell reversals occur three times more frequently in ripple wave crests than in the spaces between the crests (Sager and Kaiser 1994). In contrast, in the outer region of the fruiting body, cells move head-to-tail in narrow circular bands, with more limited side-by-side contacts, and only rarely reverse (Sager and Kaiser 1993b).

Sogaard-Andersen and Kaiser (1996) have proposed a model in which frequent head-to-tail contacts between cells induces methylation of the frzCD protein, resulting in a reduced cell-reversal frequency and thus leading to aggregation. In an analogous fashion, the limited range of side-by-side contacts experienced in an aggregating stream might promote frzCD methylation. A similar mechanism could operate within the fruiting body, where head-to-tail contacts are common and cell reversals rare. In rippling waves, extensive head-to-tail contacts would allow cells to migrate the distance of a wavelength without reversing, after which head-to-head contacts between colliding wavefronts would trigger ensemble cell reversal. If the behavior of a cell depends on contacts with particular areas of adjacent cells, then either the C-signal and/or its receptor should exhibit at least transient localization, perhaps to a cell pole. Indeed, a precedent for such a mechanism has been established in both *E. coli* and *Caulobacter crescentus*, where several proteins involved in the chemotaxis transduction pathway have been shown to be localized to a region near the cell pole (Maddock and Shapiro 1993).

The function of rippling during *Myxococcus* fruiting body morphogenesis is not known. There are at least two
possibilities. First, because rippling typically precedes fruiting body morphogenesis, ripples could pattern cell movements or cell arrangements required for formation of the fruiting body [Reichenbach 1965]. Three exist developmental conditions, such as differentiation within submerged culture [Kuner and Kaiser 1982], however, in which fruiting bodies form in the absence of rippling. Moreover, Rhie and Shimkets [1989] have isolated suppressors of the csgA mutation that can aggregate and form fruiting bodies in the absence of rippling. Thus, the spatial patterns of rippling cells need not provide a structural framework required for fruiting body construction. Furthermore, because cells do not achieve net movement during rippling, rippling per se is unlikely to drive aggregation.

Alternatively, periodic wave impacts could serve as a self-amplifying timer during development, synchronizing gene expression to the cadence of the C-signaling periodicity. Consistent with this hypothesis, when C-factor was purified from wild-type cells and added to a csgA mutant strain, the developmental rescue of the csgA cells was found to be dose dependent. At low concentrations of C-factor, csgA cells aggregated and expressed early developmental genes, whereas at higher C-factor concentrations, cells expressed late developmental genes and sporulated [Kim and Kaiser 1991]. Moreover, when measured by a csgA–lacZ transcriptional fusion, csgA transcription appears to increase during development [Hagen and Shimkets 1990]. This observation is supported further by experiments in which Li and coworkers systematically deleted progressively greater regions of the regulatory sequence upstream of the csgA gene and found a strong correlation between the levels of remaining csgA expression and the developmental competence of that deletion strain. For strains expressing low levels of csgA, development was blocked relatively early in the developmental program, whereas strains producing greater levels of csgA advanced farther into development [Li et al. 1992].

**Morphogenesis of D. discoideum**

*D. discoideum* cells undergo a developmental cycle comparable to that of *M. xanthus*. When nutrients are plentiful, these 10-μm-wide ameboid cells migrate and feed as unicellular organisms [Loomis 1982]. Upon starvation, aggregation foci emerge at sites where cells begin to spontaneously emit trains of nanomolar cAMP pulses. Collections of nearby cells respond to a pulse train by moving in traveling waves toward the focus, thus forming a 1- to 2-cm-wide aggregation territory [Alacantra and Monk 1974; Gross et al. 1976]. Aggregation is robust by 6 hr after the onset of starvation. As the aggregate forms, the extracellular CAMP concentration rises from a nanomolar to a micromolar level [Abe and Yanagisawa 1983]. Approximately 10⁶ *Dictyostelium* cells then differentiate as a multicellular assembly, first into a mound, which forms from 8 to 10 hr of development, then into a 1–2 mm tall slug-like structure, which migrates as a single entity from 16 to 18 hr of development [Devreotes 1994; Firtel 1995]. By 24 hr, a stationary spore-filled fruiting body has formed.

Cellular differentiation depends on cell-cycle position. Starving cells at an early position in their cell cycle (S and early G₂) differentiate into prestalk cells, whereas cells at later positions (mid and late G₂) form prespore cells [McDonald and Durston 1994]. Prestalk and prespore cells coaggregate randomly until the onset of slug formation, at which time they sort out into two compartments [Williams et al. 1989]. The anterior 20% of the slug contains mostly prestalk cells, whereas prespore cells are the predominant occupants of the posterior 80% [Bonner 1952; Sternfeld and David 1982]. After migrating as a multicellular structure, the slug transforms into a fruiting body in a process known as culmination. During culmination, the slug rounds up and begins to extend upward from the substratum. Prestalk cells migrate up the outer surface of the culminating aggregate, pass through a ring-like opening at the top of the culminant and enter into the center of the mound where they deposit extracellular cellulose to form a tubular sheath [Raper and Fennell 1952]. As prestalk cells enter into the stalk sheath, the sorus of the aggregate rises up from the substratum. Within the sorus, prespores differentiate into mature spores.

**Intercellular signaling**

Cells enter the aggregation phase of development when a subset of the population begins to secrete cAMP. cAMP pulses are emitted in periodic 5–7 min bursts that travel outward from the emitting cells at speeds of 100–300 μm/min [Alacantra and Monk 1974; Gross et al. 1976]. cAMP diffuses to surrounding cells, which respond both by migrating up the cAMP gradient at a speed of 10–30 μm/min and by secreting additional cAMP [Tomchik and Devreotes 1981]. As waves of diffusing cAMP propagate outward from a cAMP source, cells migrate inward to that source in periodic steps. cAMP thus serves as a propagator species, driving wave movement (Table 1). Neighboring cells are chemically coupled to one another through the diffusion of cAMP. An additional level of intercellular coordination is provided by conditioned media factor [CMF]. During wave propagation, the intercellular concentration of secreted CMF protein allows aggregating cells to monitor their cell density prior to mound formation [Gomer et al. 1991].

**Regulation of gene expression and cell differentiation**

What is the role of cAMP in regulating the cascade of differentiation pathways that ultimately leads to sporulation? Both cAMP pulse-induced and repressed gene expression has been detected in differentiating *Dictyostelium* cells. A group of environmentally responsive early genes, such as the gene encoding K5, attain high expression levels at the onset of starvation [Mann and Firtel 1989]. These starvation-specific genes are later repressed by the pulses of cAMP that course through nascent aggregates. Concurrently, several genes required for aggre-
gation undergo induction following exposure to pulsatile cAMP, including those encoding the cAMP receptor cAR1, a cAMP-dependent protein kinase, and the Ga2 subunit (Kimmel and Firtel 1991). As development proceeds, cAMP levels in the cell population reach continuously high levels, which repress these aggregation-specific genes (Mann and Firtel 1989; Schnitzler et al. 1995). Thus both the concentration and the periodicity of oscillatory cAMP exposure govern the gene expression circuitry responsible for a significant component of the developmental program (Firtel 1995).

**Intracellular transduction**

The aggregation response to cAMP is mediated by a complex signal transduction cascade (Devreotes 1994; Firtel 1995). Extracellular cAMP diffuses to the cell surface through a milieu of extracellular and membrane-bound cyclic nucleotide phosphodiesterases (PDE), which rapidly degrade proximate cAMP and thereby increase the spatial and temporal resolution of the cAMP signal (Hall et al. 1993). The spatial distribution of PDI, an extracellular cAMP phosphodiesterase inhibitor, might also serve to coordinate the formation of circular and spiral cAMP waves. A recent mathematical model of cAMP excitation kinetics has shown that an initially heterogeneous distribution of PDI may catalyze spontaneous cAMP excitation centers early in the wave formation process, while, as development proceeds, increasingly asymmetric PDI concentrations favor the transformation of circular waves into spirals (Pålsson and Cox 1996). In this scheme nascent wave patterns are stabilized later in development, as cAMP waves induce PDE synthesis while inhibiting PDI formation. Because it has been shown that PDI is not essential for aggregation (Franke et al. 1991), aggregating wave patterns are unlikely to be regulated solely by the activity of PDI. Nevertheless, PDI could function in concert with other regulatory molecules to coordinate wave shape.

Ligand binding occurs on the cell surface, where cAMP is detected by a protein, cAR1, with the characteristic seven putative transmembrane spanning domain topology found in virtually all G-protein coupled receptors (Klein et al. 1987, 1988; Neer and Clapman 1988). Three other homologous cAMP receptors (cAR2, cAR3, and cAR4) have recently been isolated and shown to be expressed transiently at different developmental stages and to bind cAMP with variable affinities (Saxe et al. 1991a,b; Johnson et al. 1992). Upon binding of cAMP to cAR1, a heterotrimeric G protein composed of a Ga, a Gb, and a Gc subunit serve in the transduction cascade. In Dictyostelium, eight Gc subunits have been found that are transiently expressed at various times in development (Pupillo et al. 1989, 1991a, 1991b; Wu and Devreotes 1991), whereas the single Gb is expressed constitutively throughout development (Lilly et al. 1988). The Ga2 subunit is maximally expressed when cAMP is secreted from the cell, where it diffuses to neighboring cells and thus propagates the cAMP wave movement (Fig. 4). In this manner the adenyl cyclase pathway serves to faithfully relay the cAMP signal throughout the aggregating population.

Because desensitization of a receptor allows a transduction system to reset its sensitivity to a level appropriate for ambient conditions, receptor adaptation is a common feature of signaling pathways responsive to a...
wide signal range. Behavioral experiments clearly show a distinct adaptive response in chemotaxing *Dictyostelium* cells moving within propagating cAMP waves. Initially, because the concentration of external cAMP rises as the front of a cAMP wave passes over a cell, the fraction of occupied cAR receptors increases as a result of cAMP binding. Ligand binding triggers the transduction cascade that ultimately results in cell movement and further cAMP secretion. However, as the stimulus persists, cells adapt and become unresponsive, though the extent of receptor occupancy may remain high. Finally, because the concentration of external cAMP falls at the rear of a cAMP wave, the fraction of occupied cAR receptors decreases and the cells undergo deadaptation [Tomchik and Devreotes 1981].

At least two parallel and interconnected cAMP transduction cascades are thought to coordinate the activities of actin and myosin proteins within the cell [Devreotes 1994; Firtel 1995]. First, ligand-bound cAR induces a receptor-coupled G protein to release its Gs-2-GTP subunit, which may then stimulate guanylyl cyclase (GC) to convert GTP to cGMP [Janssens and Van Haastert 1987; Janssens et al. 1989; Kumagai et al. 1991]. cGMP could then associate with binding proteins potentially capable of regulating myosin activity, such as a CGP-stimulated protein kinase [Wanner and Wurster 1990]. Second, bound cAR and its coupled Gs-2 subunit may activate phospholipase C (PLC), which stimulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate (IP$_3$) and diacylglycerol (DAG) [Europe-Finner et al. 1989, Drayer and Van Haastert 1992]. Disruption of the gene encoding PLC has revealed that PLC is not essential for wave propagation or later development, as a null strain was found to be developmentally competent [Drayer et al. 1994]. Nevertheless, a PLC-mediated or a PLC-independent transduction pathway with overlapping function may regulate the intracellular IP$_3$ concentration, which could ultimately result in the rapid release of Ca$^{2+}$ from the endoplasmic reticulum and other intracellular Ca$^{2+}$ storage sites, and may also trigger an increase in the intracellular cGMP concentration [Europe-Finner and Newell 1985, 1986; Woods et al. 1986].

An additional level of cAMP-mediated control of calcium metabolism is implicated by a potentially novel calcium entry pathway. The cAR1 receptor regulates a G protein-independent calcium influx [Milne et al. 1995], perhaps mediated by the activity of cAR1 kinase [Milne and Devreotes 1993; Devreotes 1994]. This calcium influx may play a role in cellular differentiation. Pseudopod cell sequester a greater concentration of Ca$^{2+}$ than prespore cells [Maeda and Maeda 1973; Schlatterer et al. 1992; Cubitt et al. 1995], and controlled increases in the intracellular Ca$^{2+}$ levels of developing cells appear to alter the ratio of pseudopod to prespore cells [Kubohara and Okamoto 1994].

Calcium-regulated actin assembly proteins direct actin polymerization and therefore drive cell movement. It is likely that the transduction cascade described above impinges on Ca$^{2+}$-sensitive enzymatic processes that regulate linear actin assembly, including proteins that direct the rate of monomer nucleation, the extent of monomer polymerizability, and the degree of filament severing [Tsukita et al. 1989; Maekawa et al. 1989]. Moreover, coordination of the three-dimensional actin filament architecture is controlled by a set of partially redundant *Dictyostelium* actin-binding proteins, including two 30-kD proteins and one 120-kD protein found to organize filaments into tight and loose bundles, respectively [Stossel 1989; Witke et al. 1992]. Ultimately, nascent F-actin filaments provide cytoskeletal substrates for polarized pseudopodial formations. Localized filopodial growth initiates directed cell movement, which is reinforced by the flow of cortical actin.

A multiplicity of mechanisms coordinates both myosin I and myosin II activity. Elimination of a single member of the myosin I family significantly alters cell motility [Titus et al. 1993], arguing that this protein directs cell movements in vivo. Moreover, myosin II is required for normal cell movement, as demonstrated by the reduced motility phenotypes of myosin II null cells [De Lozanne and Spudich 1987, Knecht and Loomis 1987]. It is likely that a set of cGMP-stimulated protein kinases act as to regulate myosin function, as three phosphorylatable threonine residues within the myosin II heavy chains have been shown to function as regulatory sites in vivo [Egelhoff et al. 1993; Gerisch et al. 1993].

**Cell movements within cAMP waves**

Changes in individual cell shapes and locomotory patterns drive aggregating wave movements [Chen et al. 1996]. While the concentration of cAMP is rising in a wave, cells exhibit a decreased frequency of lateral pseudopod formation as they elongate and migrate toward the cAMP source. As the concentration of the cAMP peaks, the cells reduce their rate of pseudopod formation and maintain a more fixed shape. When cells perceive a decreasing cAMP concentration, they again initiate pseudopod formation, but in a random manner and without net movement [Alacantra and Monk 1974; Varnum et al. 1985, Wessels et al. 1992; Siegert and Weijer 1993a].

**cAMP and directed cell movement**

The aggregation of differentiating *Dictyostelium* cells results directly from the inward movements of cells responding to outward-moving cAMP pulses emanating from a central spatial locus [Tomchik and Devreotes 1981]. Because successful mound formation and cellular differentiation depend upon the high density of cell–cell contacts brought about by aggregating wave movements [Gerisch 1986, Desbarats et al. 1994], cAMP wave propagation is required for proper *Dictyostelium* development. Furthermore, cAMP waves direct cell behavior in later morphogenesis, that is, at the mound stage of development. Labeled anterior-like cells in the prespore region of the slug have been shown to exhibit periodic cell migrations, in which cells move in concentric rings and
spirals [Siegert and Weijer 1993a, 1995]. These patterns of movement argue that cAMP signaling waves exist in the slug in at least two distinct forms. In the prestalk region, signaling may take the form of a twisted scroll wave, whereas in the prespore zone, the cAMP signal appears to propagate as a planar wave. In spite of the potential complexity of this signaling architecture, the temporal patterns of cAMP exposure per cell may follow a relatively simple periodic function. A model of wave propagation in the slug shows that different cells within the prestalk zone could undergo oscillations at different phases and amplitudes. This information could be used to convert periodic signals into stable positional cues leading to spatially restricted cellular differentiation [Bretschneider et al. 1995].

Comparison of traveling wave movements in Myxococcus and Dictyostelium

Both Dictyostelium and Myxococcus cells self-organize into bands that move in pulsatile, traveling waves. In Dictyostelium, cells respond to a pulse of cAMP both by migrating toward the source of the signal emission and by releasing cAMP into the medium, generating a signal relay that propagates the wave movements [Tomchik and Devreotes 1981]. For Myxococcus cells, in vitro exposure to C-factor both modulates cell movements [Sager and Kaiser 1994] and increases transcription of a csgA–lacZ gene fusion [a twofold increase was noted at 40 hr of development; Kim and Kaiser 1991]. If C-factor-directed cell movements cause cells to form arrangements that favor C-signaling, C-factor production could be autocontrolled. Thus in both these organisms, traveling waves might be directed by the activity of a signaling molecule whose production is positively autoregulated.

There are however at least two significant differences in the mechanisms of wave propagation for developing Myxococcus and Dictyostelium cells. First, Myxococcus rippling wavefronts intersect and migrate past one another without generating wave interference patterns [Fig. 1A; Shimkets and Kaiser 1982; Sager and Kaiser 1994]. In contrast, Dictyostelium waves annihilate one another on impact, so that countermigrating wavefronts cannot advance past one another [Fig. 1C; Tomchik and Devreotes 1981]. This topological difference can be understood in terms of the adaptation states of the rippling cells. After cells experience a wave of cAMP, the cAMP transduction pathway becomes desensitized. During this transient refractory period, the cells on the leading edge of a wave remain adapted and cannot propagate the cAMP signal. When Dictyostelium waves intersect head on, neither wave can advance [Tomchik and Devreotes 1981]. This because in Myxococcus C-signaling wavefronts appear to repeatedly reflect off one another, cells do not appear to adapt to the C-factor. In Myxococcus, the refractory period for cell–cell excitation may be set by the distance a cell travels before encountering a particular intercellular contact (Table 1).

Second, in Myxococcus, the wavelength of a particular culture is constant throughout the culture [Reichenbach 1965; Shimkets and Kaiser 1982, Sager and Kaiser 1994]. This constancy suggests that a field of rippling Myxococcus cells represents a relatively stable excitable medium. In contrast, the wavelength of Dictyostelium waves can vary within the same culture [Tomchik and Devreotes 1981; Fig. 1, cf. A and C]. Although a particular wave has a constant velocity, the velocity of successive waves emanating from the same organizing center progressively decreases, wave velocity has been shown to decrease almost twofold after the evolution of only 10 cAMP waves within an aggregation field [Gross et al. 1976]. Thus older, faster bands are spaced further apart than younger, slower bands, ultimately resulting in a threefold variation in wavelength within the same culture [Tomchik and Devreotes 1981; Fig. 1C]. The mechanism underlying this change is not well understood, but may be related to wave speed dispersion, the dependence of wave speed on the relative excitability of the medium. Because a relatively short period might not allow the medium sufficient time to recover to full excitability before propagation of the next wave, a declining period is usually associated with a reduced wave speed [Tyson and Keener 1988; Gerhardt et al. 1990]. How might wave speed dispersion help to explain the variation in wave spacing? For Dictyostelium, the aggregation function of wave propagation described above results in a progressive increase in cell density at the aggregation center. A heightened density may augment the excitability of the cells within the center, increasing the frequency and thus decreasing the period of the wave movements in this region. This decline in period might alter the dispersion relation for the chemotaxing cells and lower the propagation speed of the cAMP wave [Höfer et al. 1995; Goldstein 1996].

Summary

The ubiquity of pattern-forming chemical reactions argues that relatively simple autocatalytic systems can form complex and dynamic morphological structures such as traveling waves [Field and Berger 1985]. Thus the patterns of multicellular wave movements found in developmental fields need not result from the actions of intrinsically complex regulatory networks. Rather, relatively simple signaling reactions can in many cases drive wave formation. The signal molecule may be cell associated, as in Myxococcus [Shimkets and Raifee 1990] or freely diffusible, as in Dictyostelium [Tomchik and Devreotes 1981]. In the wave-forming systems described in this article, pairs of propagator and controller species act as pattern perturbing and restorative forces, respectively (Table 1), and together determine the morphological properties of the wave (Table 2).

The extent to which diffusion plays a role in the wave propagation mechanism delineates two major classes of waves. Trigger waves are formed through the coupling of the autocatalytic production of a propagating signal and its diffusion into neighboring regions of an excitable medium. In contrast, phase waves do not rely on diffusion for propagation, instead depending on intrinsic phase
Table 2. A comparison of the patterns of movement for rippling Myxococcus xanthus cells, waves of aggregating Dictyostelium discoideum cells, and oxidation bands of the ferroin-catalyzed Belousov-Zhabotinsky (BZ) reaction

<table>
<thead>
<tr>
<th>Wave system</th>
<th>Wave velocity (mm/min)</th>
<th>Wave period [min]</th>
<th>Wavcrest width [mm]</th>
<th>Wavelength [mm]</th>
<th>Wave type</th>
<th>Impact effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxococcus xanthusb</td>
<td>0.0085</td>
<td>7.5</td>
<td>0.01</td>
<td>0.065</td>
<td>phase</td>
<td>no change</td>
</tr>
<tr>
<td>Dictyostelium discoideumc</td>
<td>0.3</td>
<td>10</td>
<td>1.0</td>
<td>3.0</td>
<td>trigger</td>
<td>annihilation</td>
</tr>
<tr>
<td>BZ reactiond</td>
<td>4.2</td>
<td>0.3</td>
<td>0.34</td>
<td>1.24</td>
<td>phase + trigger</td>
<td>annihilation</td>
</tr>
</tbody>
</table>

*Morphology after impact of counter-migrating waves.
Sager and Kaiser [1994].
Tomchik and Devreotes [1981].
Müller and Plesser [1995].

gradients within the medium. Both types of waves may be present within the same morphogenetic field. The ferroin-catalyzed BZ waves are composed of both an oxidizing trigger wave at the wavefront and a reducing phase wave at the waveback [Tyson and Fife 1980; Showell 1981].

The appearance of traveling waves can be generated solely from the activity of phase waves. The wave-reflection model of Myxococcus rippling argues for pseudotraveling phase waves, in which the differential timing gradient of cell-cell impacts promotes a reflection in the C-signaling wave shape. In contrast, the wave behavior of an aggregating Dictyostelium culture appears to be significantly more complex than that of rippling Myxococcus. Dictyostelium trigger waves display anisotropic excitability, where the aggregating cells assume different movement and cAMP secretion patterns as a function of distance from an aggregation center. Moreover, Dictyostelium aggregation may provide the first example of an excitable medium that allows self-modulation of its excitability during active wave propagation [Höfer et al. 1995].

Self-amplified production of a gene product frequently serves as a choice point in a developmental pathway, as exemplified by the critical switch roles of a diverse set of genes, including the Drosophila Sex-lethal (Sxl) gene [Bell et al. 1988], the nuclear proto-oncogene c-myb (Nicolaides et al. 1991), and the phage λ ci gene [Ptashne 1986]. Each of these molecules functions by means of positive feedback loops at critical branch points in their respective differentiation programs. Indeed, the C-signaling and cAMP transduction pathways may both serve as autoregulatory switches in their respective developmental circuits. Their primary function might be to promote commitment to a developmental pathway. In this case, utilization of their wave-forming properties during development may have arisen after their initial selection as robust molecular switches. Alternatively, the evolutionary selection of the C-signaling and cAMP transduction pathways as components of switching circuits may have resulted in part from their intrinsic organizing properties.

There are several potential advantages for the use of wave propagation during multicellular development. First, wave formation in both Myxococcus and Dictyostelium relies on the tight coupling of intercellular signaling to cell movement. This coupling permits the simultaneous regulation of both macroscopic cell assembly and cellular differentiation, and might allow the evolution of finely tuned developmental circuits that depend on a dialog between these processes. Furthermore, because the movement of a wave takes place in space, wave migration could lead to the persistence of a periodic pattern of differentiating cells and therefore promote the organized spatial development of a tissue or organ. Finally, because the movement of a wave takes place in time, a robust developmental timer could be set by the time needed for a cell to travel a certain length [such as the distance of a wavelength]. Given the precedents set in Myxococcus and Dictyostelium development, it is likely that a variety of intercellular signals form traveling waves during metazoan embryogenesis.

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Sager


Sager


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