REVIEW

Integration of signaling information in controlling cell-fate decisions in *Dictyostelium*

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Over the last few years, a combination of genetic, molecular, and biochemical approaches has led to significant new insights into the regulation of development in *Dictyostelium* and has uncovered new pathways that may also control developmental decisions in other systems. In *Dictyostelium*, the vegetative growth and the multicellular developmental portions of the life cycle are separated; wild-type *Dictyostelium* strains are haploid, and multicellular development, which is initiated by starvation, takes place in the absence of cell division. Thus, many genes that are essential for the multicellular phase have no effect on growth, facilitating genetic analysis of gene products essential for multicellular development. The general developmental program of *Dictyostelium* is depicted in Figure 1.

Dictyostelium is an ideal organism for studying how signaling pathways are integrated to control morphogenesis and gene regulation, and much of the recent progress in understanding the control of multicellular development in *Dictyostelium* is a result of linking the various components into regulatory networks. The identification and functional analysis of stage and cell-type-specific G proteins, serpentine (G protein-coupled) receptors, the transcription factor GBF (G-box-binding factor), MAP (mitogen-activated protein) kinases, PKA (protein kinase A), GSK-3 (glycogen synthase kinase-3) (the latter two control cell-fate decisions in *Drosophila*), and other signaling molecules common to all eukaryotes have provided basic, new insights into control of these processes in *Dictyostelium* and other systems. In addition, novel pathways have been identified that are regulated by extracellular cAMP functioning through serpentine receptors to activate distinctly different developmental pathways, depending on whether the cells perceive a pulsatile or sustained signal. This has led to an initial understanding of how signaling and transcriptional events are integrated to control developmental timing and how cell-type specification is regulated by these factors and extracellular morphogens.

This review examines these recent findings and describes how they have led to a new understanding of the molecular mechanisms regulating the developmental de-

cisions in *Dictyostelium* and their relationship to developmental decisions in other organisms.

Formation of the multicellular organism

Multicellular development is initiated several hours after removal of the food source, when a small number of cells within the population emit an extracellular pulse of cAMP. Cells respond by chemotaxis toward the signal and activation of adenylyl cyclase (AC) to produce more cAMP, which is secreted/released (Devreotes 1994). Cyclic AMP binding to receptors on outlying cells relays the signal, whereas binding to additional receptors on the activated cell amplifies the signal. Like receptor-coupled pathways in other systems, the responses are transient; they adapt within 1–2 min, and the cells regain responsiveness to cAMP in 5–6 min. About 20–30 pulses (cycles of activation and adaptation of the pathways) are required for an aggregation domain to produce a mound. In addition to regulating the relay of the cAMP signal and chemotaxis, the cAMP pulses also induce expression of genes required for aggregation (pulse-induced genes), as described below.

Adaptation of the aggregation responses is crucial because this provides directionality to the chemotactic movement toward the aggregation center. The rapid adaptation ensures that cells respond only to cAMP from the direction of the aggregation center and not to cAMP secreted by outlying cells (Tomchik and Devreotes 1981; Van Haastert et al. 1987). As long as cAMP is present, the pathways remain adapted. To remove extracellular cAMP, aggregation-stage *Dictyostelium* cells express an extracellular phosphodiesterase (PDE), which rapidly hydrolyzes the extracellular cAMP (Franke and Kessin 1992). The level of active PDE is tightly regulated both at the level of transcription and via a phosphodiesterase inhibitor (PDI, which binds to and inhibits the PDE), because high levels of PDE would interfere with diffusion of cAMP to adjacent cells and low levels would result in the sustained presence of cAMP and prolonged adaptation. High levels of cAMP stimulate PDE expression and inhibit the expression of PDI, whereas low lev-

Figure 1. The developmental cycle is shown. The first panel shows spore germination. A dark-field image of an aggregation domain is shown at 6 hr. Developing mounds are seen at 8 hr and 10 hr, and a tipped aggregate at 12 hr. At 14 hr, a finger has formed that falls over to become the slug (16 hr). Culmination ensues at 18 hr, and a mature fruiting body is formed by 24 hr. The developmental phases are shown with the periodicity of cAMP production. (Bottom) The developmental kinetics of gene expression, emphasizing the transcriptional changes at the time of mound formation. At the initiation of development by starvation, a set of genes are induced that are then repressed by pulses of cAMP. These pulses induce another set of genes (pulse-induced genes) that play essential roles during aggregation. As the mound forms, these pulse-induced genes are repressed and the developmental cascade leading to cell-type differentiation is initiated by the induction of GBF followed by the postaggregative or primary-response genes and the cell-type-specific genes. (See text for details.)

els of cAMP stimulate PDI expression (Franke and Kessin 1992). The regulatory gene products discussed in this review are described in Table 1.

A series of integrated signaling pathways regulates aggregation

Analysis of the signaling pathways that regulate aggregation in *Dictyostelium* provides general insight into eukaryotic receptor-regulated pathways, including chemotaxis in mammalian cells (Devreotes and Zigmond 1988). cAMP binds to classic serpentine cell-surface receptors (cAMP receptors or cARs) that couple to the heterotrimeric G protein containing the Gα subunit Gα2 (Fig. 2). Three second-messenger pathways are activated: guanylyl cyclase (GC), phospholipase C (PLC), and AC (Devreotes 1994). AC activity peaks at ~90 sec and then adapts, whereas GC and PLC peak and adapt more rapidly (10 and 7 sec, respectively). Continuous fluxes of cAMP cause a single, transient activation of these pathways, followed by the eventual down-regulation of the receptors. Analysis of null strains indicates that the receptor and the G protein are essential for these responses in vivo (Kumagai et al. 1989, 1991; Kesbeke et al. 1990; Sun and Devreotes 1991; Wu et al. 1995). cAR1, the most abundant cAR during aggregation, has a high affinity for cAMP and is able to respond to the low concentrations of cAMP present during the early stages (Johnson et al. 1992; Insall et al. 1994b). cAR3, a lower affinity cAR, can replace cAR1; however, higher concentrations of cAMP are required to activate the responses (Insall et al. 1994a). This indicates that multiple cARs couple to Gα2 and can regulate the same signaling pathways.

The activation of AC is regulated via a fairly complex pathway. The AC expressed during aggregation (ACA)

has the same predicted structure as mammalian ACs (Pitt et al. 1992; Tang and Gilman 1992) and is thought to be directly regulated by the Gβγ subunit rather than a specific Gα subunit. Several lines of evidence support this conclusion. In vivo, cAMP activation of AC requires Gα2, whereas in vitro, AC can be activated in gα2 but not gβ null cells by GTPγS (Kesbeke et al. 1988; Kumagai et al. 1991; Wu et al. 1995). Overexpression of Gα2 suppresses the activation of AC but stimulates the activation of GC (Okaichi et al. 1992). At least two other components are required for AC activation in *Dictyostelium*: the MAP kinase ERK2 and a cytosolic protein, CRAC (Lilly and Devreotes 1994; Segall et al. 1995). CRAC has a pleckstrin domain, suggesting that it interacts with another protein, possibly the Gβ subunit (Insall et al. 1994a; Touhara et al. 1994). MAP kinases are known to be activated through both receptor tyrosine kinases and G protein-coupled receptors (Davis 1993; Errede and Levin 1993; Blumer and Johnson 1994) but have not been known previously to be required for the activation of AC.

Activation of GC and production of cGMP have been linked to chemotaxis and the membrane-associated polymerization of actin and myosin, similar to pathways mediating chemotaxis in mammalian cells (McRobbie and Newell 1984; Devreotes and Zigmond 1988; Liu et al. 1993). Mutations affecting the GC/cGMP pathway result in cells that are not capable of chemotaxis or that show altered chemotaxis but activate AC normally (Newell and Liu 1992; Kuwayama et al. 1993). The gene encoding PLC has been disrupted, but there is no developmental phenotype, suggesting that PLC activity is not required for aggregation or other stages in development (Drayer et al. 1994). For a more detailed analysis, the reader is referred to a recent review of this pathway (Devreotes 1994).

Table 1. *Regulatory function of cloned genes described in text*

Aggregation	
cAR1	high-affinity cAMP receptor; required for all aggregation responses to nanomolar pulses of cAMP
cAR3	lower affinity cAMP receptor; can mediate aggregation-stage responses but only to higher levels of cAMP
Gα2	Gα subunit; couples to cAR1; required for all cAMP-aggregation responses
CRAC	cytosolic activator of adenylyl cyclase (AC), required for G-protein activation of AC
Gβ	Gβ subunit; required for all cAMP-aggregation and folate-mediated responses except Ca ²⁺ influx and activation of the GBF pathway; no activation of AC, guanylyl cyclase (GC), or chemotaxis
ERK2	MAP kinase; required for AC stimulation
ACA	adenylyl cyclase (AC) gene expressed during aggregation; required for production of cAMP and signal relay
CMF	conditioned medium factor; senses cell density; required for aggregation
PKAcat	catalytic subunit of cAMP-dependent protein kinase (PKA); required for AC activity; also required for other processes in aggregation
GC	guanylyl cyclase (GC); activity involved in actin/myosin mobilization and chemotaxis in response to cAMP, folate, and other chemoattractants
PDE	extracellular phosphodiesterase; required for regulating cAMP levels; overexpression or a null mutant blocks aggregation
ERK1	MAP kinase; overexpression of ERK1 in a null mutation of the protein-tyrosine phosphatase PTP2 results in very large aggregation streams that split into very small aggregates that arrest
PTP2	protein-tyrosine phosphatase; null cells produce larger aggregation streams and larger slugs; phenotype suggests that PTP2 affects signal relay
PTP1	protein-tyrosine phosphatase; cells overexpressing very high levels of PTP1 do not aggregate
Mound/tip	
GBF	transcription factor required for all late gene expression; activated by extracellular cAMP
cAR1/cAR3	required for cAMP activation of GBF function and postaggregative development
cAR2	cAMP receptor; induced at mound stage and required for tip formation; null cells overexpress prespore genes
LagC	putative cell-surface ligand; required for prespore and prestalk cell differentiation
PKAcat	required for prestalk and prespore gene expression
rasD	involved in tip formation
Gα4	couples to folate receptors; null cells arrest at finger stage; required for prespore/spore pathway
PTP1	protein-tyrosine phosphatase; overexpression of PTP1 results in aggregates with multiple tips
PDE	phosphodiesterase; overexpression blocks tip formation; indicates importance of cAMP in this process
PTP2	protein-tyrosine phosphatase; overexpression of PTP2 results in aggregates that arrest at the mound stage
Prespore cells	
GSK-3	glycogen synthase kinase-3; required for prespore cell formation
Gα4	required for normal expression of prespore genes and spore formation
cAR2/cAR4	null strains for either receptor show overexpression of prespore markers and a reduced expression of prestalk markers; phenotype suggests that it is involved in prestalk differentiation and inhibiting prespore cell differentiation
Prestalk cells	
GSK-3	negative regulator of prestalk B differentiation
cAR2/cAR4	see above
Slug	
Gα1	Gα-subunit; overexpression results in abnormal slug morphology and reduced/no prestalk B cell gene expression; negative regulator of prestalk B differentiation?
Gα7	Gα-subunit; overexpression results in abnormal slug morphology and culmination
ERK1	overexpression alters slug morphology and culmination; increased number of rear guard cells
PTP1	overexpression of PTP1 results in slugs with large, bulbous anteriors
Culmination	
Gα2	expression of a dominant-negative Gα2 results in abnormal stalk morphogenesis during culmination; indicates a role for both cAMP receptors and G-protein-coupled pathways
Gα1	expression of a constitutively active Gα1-subunit results in abnormal morphogenesis during culmination; activity required for the rise in 1,2-diacylglycerol levels associated with the induction of culmination
cAR4	cAMP receptor; required for proper culmination and spatial patterning; null cells overexpress prespore genes
PKAcat	required for prestalk → stalk and prespore → spore differentiation; overexpression in prespore cells induces spore differentiation very early in development; preferentially expressed in prestalk A cells in slug and prespore cells in culminant

Aggregation competence, developmental timing, and regulation of gene expression

Although addition of cAMP to washed, vegetative cells results in the stimulation of AC and GC, the levels of

activation are low, resulting from a low level of the essential signaling components at this stage of development, and there is no effective chemotaxis of the cells to cAMP (Kesbeke et al. 1986; Okaichi et al. 1992). Genes encoding several of these components (cAR1, Gα2, PKA)

Figure 2. cAMP receptor-mediated pathways during aggregation and multicellular development. During aggregation, cAMP binds to cAR1 and activates GC, PLC, AC, and pulse-induced gene expression. The pathway is drawn with a direct coupling of GC and PLC to the G protein containing $G\alpha_2$, although this has not been proven. AC is thought to be activated by $G\beta\gamma$, and this activation is known to require CRAC, the cytosolic activator of AC, and the MAP kinase ERK2. In vivo, all responses require both the $G\beta$ subunit and $G\alpha_2$. In addition, cAMP binding results in a Ca^{2+} influx, which is G protein-independent. The function of this pathway is not known. In the multicellular stages, activation of late gene expression occurs through cARs but is independent of G proteins. cAMP response pathways similar to those present during aggregation may regulate morphogenesis during tip formation and culmination through $G\alpha_2$. Other $G\alpha$ subunits and other G protein-independent pathways may interact with cAR2, cAR4, and other serpentine receptors. Data are from Schnitzler et al. (1995).

belong to the "pulse-induced" class of genes (Kimmel and Firtel 1991). Low-level transcription of the pulse-induced genes is activated by starvation and requires a secreted protein factor, conditioned media factor (CMF), which functions as a density-sensing factor to ensure that the concentration of cells is sufficient for successful chemotaxis and aggregation (Gomer et al. 1991; Yuen et al. 1991). This starvation-mediated response leads to a sufficient level of receptors and other components to initiate signaling. As waves of cAMP move through the population, expression of this class of genes is rapidly induced to a significantly higher level. The pathway shows positive autoregulation in that proteins that are induced are required for their own induction (e.g., cAR1 and $G\alpha_2$). As with the other aggregation-stage pathways, a continuous cAMP signal blocks this induction (Mann and Firtel 1989). If the pulsatile cAMP signal is switched to a high, continuous signal, the pulse-induced genes are rapidly repressed (Mann and Firtel 1989; Schnitzler et al. 1995). The timing of pulse-induced gene expression is shown in Figure 1.

The ability to mimic the in vivo oscillatory pattern of cAMP by adding exogenous cAMP to cell suspension cultures has allowed a determination of which components of the aggregation-response pathways are required for pulse-induced gene expression. Cells in which AC activation is defective, such as cAMP-dependent PKA, CRAC, or ACA null cells, cannot aggregate by themselves but will respond chemotactically to cAMP pulses and induce early gene expression (Mann and Firtel 1991; Pitt et al. 1992; Insall et al. 1994a). These cells can aggregate with wild-type cells to form a chimeric organism, and some will participate in spore formation, although at a low frequency. Together, these results indi-

cate that intracellular cAMP is not required for pulse-induced gene induction. As with other aggregation-stage responses, either cAR1 or cAR3 is required for this induction (Soede et al. 1994). We expect that the pathway will involve the integration of multiple, transient responses over a period of time. Such a pathway has been defined in mammalian neuronal cell lines in which Ca^{2+} /calmodulin kinase is activated by multiple, transient increases in intracellular Ca^{2+} that, on reaching a threshold concentration, activate the enzyme to a high level (Meyer et al. 1994).

Spatial localization of cell types—cell sorting and morphogenesis

As the mound forms, there is a developmental switch from the formation of a multicellular organism to the induction of the specific cell types that are the precursors of cells found within the mature fruiting body. This switch is marked molecularly by the induction of post-aggregative and then cell-type-specific genes (Fig. 1). A "fate map" has been constructed throughout the multicellular stages using *lacZ* and β -glucuronidase (GUS) reporter constructs driven by promoters for a number of these genes (Fig. 3; Gomer et al. 1986b; Dingermann et al. 1989; Haberstroh and Firtel 1990; Jermyn and Williams 1991; Esch et al. 1992; Early et al. 1993). Early studies defined three major cell types in the migrating slug: prestalk cells, preferentially located in the anterior 15% of the slug; prespore cells, located in the posterior 75%; and anterior-like cells (ALCs), a population of cells (5%–10%) scattered throughout the organism with some properties of prestalk cells (Loomis 1982; Sternfeld and David 1982). Analysis of promoters for the prestalk genes *ecmA* and *ecmB* has now defined multiple classes of

Figure 3. Fate map of cell types during *Dictyostelium* development. (A) The formation of the initial prestalk and prespore patterns as the mound is forming. (Left) The patterning as cells are still moving into the mound and as the tip is forming. The prestalk cells become localized in the tip by differential sorting (see text) and can be seen to move in a spiral pattern, presumably following the spiral waves of cAMP. The localization of the prespore cells is also shown. The unlabeled, darker region represents cells that have not expressed cell-type-specific markers. (Right) The initial pattern of prestalk and prespore cells in the tipped aggregate and the presence of ALCs (small circles) in the organism. The cartoon is derived from the analysis of the whole *ecmA* (prestalk A and O, defined here using the original terminology of prestalk A cells), *ecmB* (prestalk B), and the *SP60* (prespore) promoters. Results describing the initial patterning of prestalk A compared with prestalk O cells have not been published. (B, top) The patterning of the cell types in the slug and the movement and interconversion of prestalk and ALCs (see text; Abe et al. 1994 for details). (C) The patterning during culmination. The drawings in part were a gift of J. Williams (MRC Laboratory for Molecular Biology, London, UK).

prestalk cells (Williams et al. 1993b). The ALC population is also complex, with different subpopulations expressing different combinations of *ecmA* and *ecmB*, as well as some regulatory genes including *ERK1*, *PTP1*, *PTP2*, and *Gα4* (see below; Jermyn and Williams 1991; Howard et al. 1992, 1994; Gaskins et al. 1994; Hadwiger et al. 1994). The functions of these genes are described

below and in Table 1. A listing of the cell-type-specific markers is presented in Table 2.

Prespore-specific promoters (see Table 2) show a homogeneous expression over the posterior ~85% of the slug (Dingermann et al. 1989; Haberstroh and Firtel 1990; Fosnaugh and Loomis 1993; Powell-Coffman and Firtel 1994). However, various *CotC/SP60* promoter deletions show anterior-to-posterior or posterior-to-anterior gradients of expression, suggesting that differences exist within the prespore domain (Haberstroh and Firtel 1990; Haberstroh et al. 1991). Moreover, when cells from the back, middle, and front of the prespore region were marked and transplanted into other slugs, differences in behavior of the cells were observed (Buhl et al. 1993). Taken together, these results suggest the existence of gradients of morphogens within the prespore domain that differentially affect promoter elements and lead to the behavioral differences of the cells.

The spatial patterning of prestalk and prespore cells within the mound arises from the sorting of the prestalk cells to the presumptive tip of the mound (Williams et al. 1989; Esch and Firtel 1991; Williams and Jermyn 1991). This appears to be mediated by cAMP using pathways that may be identical to those that mediate chemotaxis during aggregation, with prestalk cells having an inherently higher rate of chemotactic movement than prespore cells (Mee et al. 1986). Inversion of the putative apical-to-basal cAMP gradient (achieved by placing mounds on agar having a high concentration of cAMP) results in sorting of prestalk cells to the base, whereas disruption of the gradient by overexpressing PDE from its own prestalk-specific late promoter (and thus reducing cAMP around these cells) blocks tip formation (Traynor et al. 1992; Hall et al. 1993). Strains lacking the gene encoding the cAMP receptor *cAR2*, which is preferentially expressed in prestalk cells, arrest at the mound stage, with a few aggregates developing a tip, suggesting that *cAR2* mediates cell sorting during tip formation (Saxe et al. 1993).

The apical tip functions as an "organizer" that controls subsequent morphogenesis and is the source of the cAMP wave that is thought to initiate at the tip and move toward the posterior (Siegert and Weijer 1991, 1992). It acts as a center for the sorting of the prestalk cells and inhibits other oscillators from forming by down-regulating the cAMP relay within the remainder of the aggregate (Wang and Schaap 1985). Wild-type strains form only a single tip. Mutant strains that initially form multiple tips either developmentally arrest, as observed for cells overexpressing the constitutively active *RasD(G12T)* mutation (Reymond et al. 1986), or each tip "organizes" an independent organism that separates and migrates apart, as in the case of strains that overexpress the protein-tyrosine phosphatase *PTP1* (Howard et al. 1992).

As the mound forms, there is an increase in the level of intercellular cAMP, which adapts aggregation-stage pathways mediated through the *cAR1* high-affinity receptor (see above). How is an oscillator maintained if these pathways are adapted by the high level of cAMP?

Table 2. Cell type-specific markers described in text

Prestalk	
<i>ecmA</i>	extracellular matrix protein; prestalk A/O, ALCs
<i>ecmB</i>	extracellular matrix protein; expressed in prestalk B cells and ALCs; a deletion of <i>ecmB</i> promoter (<i>ecmB</i> Δ89) is expressed specifically in stalk cells
Gα1	Gα-subunit; shows same pattern of expression as <i>ecmB</i>
ALCs	
Gα4	Gα-subunit
PTP1, PTP2	protein-tyrosine phosphatases; PTP1 and PTP2 are distinct and have different developmental functions
ERK1	MAP kinase
<i>ecmA/ecmB</i>	<i>ecmA</i> and <i>ecmB</i> encode extracellular matrix proteins; they are also markers for prestalk A/O and prestalk B cells, respectively
Gα1	Gα1 shows a similar pattern of expression as <i>ecmB</i>
Prespore	
SP60/CotC	spore coat protein
SP70/CotB	spore coat protein
PspB	spore coat protein
PspA	cell-surface-associated protein
Prestalk-enriched	
CP2	CP2/pst-cathepsin; encodes a cysteine protease
<i>rasD</i>	encodes one of five Ras proteins
cAR1	cAMP receptor; the cAR1 late promoter is preferentially expressed in prestalk cells
cAR2/4	cAMP receptors; both receptors are induced after mound formation; cAR2 is expressed before cAR4 and from genetic analysis functions before cAR4; both are expressed at a significantly higher level in prestalk than prespore cells
Spore	
<i>spiA</i>	spore coat protein; induced at the onset of prespore → spore differentiation during culmination

This dilemma is solved by preferentially expressing essential components of the signaling pathway in the anterior region of the slug. cAR2 expression is induced at the mound stage and is preferentially expressed in prestalk cells. cAR2 is a very low-affinity receptor, suited to mediating oscillatory pathways when cAMP levels are high (Johnson et al. 1992). Another essential component needed for oscillator function is PDE, which is necessary to hydrolyze the extracellular cAMP. During the multicellular stages, PDE is preferentially expressed in the anterior prestalk A cells, in a location necessary to maximally reduce the cAMP levels in the tip (Hall et al. 1993).

One of the striking aspects of the spatial pattern within the slug is the sharp boundary between the prestalk and prespore regions in the absence of an extracellular matrix to form a barrier. In *Dictyostelium*, cell-type differentiation is plastic, similar to the situation for cells in some metazoan embryos (Davidson 1986), but more extreme, with the prestalk and prespore cells not being fully committed to form stalk cells and spores until culmination. Prestalk cells can differentiate into stalk cells and vice versa under specific conditions, and cells can respond to changes in nutrient availability by reentering the cell cycle (Loomis 1982). Moreover, in studies in which prespore cells are precociously induced to form spores, new prespore cells arise from the remaining prestalk and ALC populations (Mann and Firtel 1993; Mann et al. 1994). During normal development, there is

some back-and-forth movement between the prestalk, ALC, and prespore populations with limited dedifferentiation and redifferentiation, highlighting both the plasticity of the cells and the absence of a barrier between the prestalk and prespore zones (Abe et al. 1994). This overall plasticity allows organisms of varying sizes to have the same spatial patterning. The pathway that regulates the prestalk/prespore boundary is not known; however, cells lacking cAR4, which is expressed most strongly in the anterior prestalk cells, show a partial breakdown of the boundary and the presence of significant numbers of prespore cells in the anterior region (Louis et al. 1994).

The role of the cell cycle in directing prestalk and prespore pathways

A number of studies indicate that the position of a cell in the cell cycle at the time of starvation plays an important role in controlling the initial decision whether a cell differentiates into a prestalk or prespore cell. As in *Schizosaccharomyces pombe*, the cell cycle in *Dictyostelium* lacks a G₁ phase (Weijer et al. 1984a). Individual cells starved early in the cell cycle (S and early G₂) have a propensity to differentiate into prestalk cells, whereas cells starved later in G₂ tend to differentiate into prespore cells (Weijer et al. 1984b; Gomer and Firtel 1987; Maeda 1993; Araki et al. 1994). The prestalk/prespore ratio obtained using cell culture assays is similar to that

found in the slug. However, synchronized early cells or late cells plated for development form normally proportioned aggregates, again highlighting the plasticity of *Dictyostelium* development. S- and early G₂-phase cells have a higher concentration of many of the signaling components needed for aggregation and are the first to initiate cAMP pulsing within an unsynchronized population (McDonald 1986; Wang et al. 1988). Cells starved in late G₂ can also initiate cAMP signaling, but they require several additional hours of starvation before they initiate this process.

Differential transcriptional regulation of the signaling components during the cell cycle in vegetative cells might be the molecular mechanism for the initial determination of whether a cell differentiates into a prestalk or prespore cell. Gα1, RasD, and the protein tyrosine phosphatase PTP2 affect aggregation-stage responses. They are expressed at low levels in vegetative cells and are preferentially expressed in a prestalk-enriched or ALC pattern during the multicellular stages (Esch and Firtel 1991; Howard et al. 1992, 1994; Dharmawardhane et al. 1994). Promoter/*lacZ* reporter studies of these genes show that only ~10%–15% of the vegetative cells stain strongly at any one time. For *rasD*, in which expression has been examined in cell cycle-synchronized populations, the fraction of cells that stain changes throughout the cell cycle (Esch 1991). Approximately 50% of the cells stain early in the cell cycle, whereas the fraction decreases to <5% for late G₂ cells. Thus, the level of one or more of these or other components as cells are starved may control the cell cycle effect on cell-type differentiation.

The transcription factor GBF regulates the developmental switch from aggregation to cell-type differentiation

A developmental switch from aggregation to cell-type differentiation is induced late during aggregation. As the mound forms, cAMP levels start to rise (Abe and Yanagisawa 1983). This inhibits the expression of the aggregation-stage, pulse-induced genes by adapting the pathways (see above) and activates a cascade that results in the induction of postaggregative and cell-type-specific genes, prestalk and prespore cell differentiation, and morphogenesis (Town and Gross 1978; Mehdy et al. 1983; Kimmel and Firtel 1991; Williams 1991; Schnitzler et al. 1994). This developmental switch is regulated by a change in the cAMP receptor-mediated signaling pathway from one that is regulated by nanomolar pulses of cAMP during aggregation to one that is controlled by a higher, more continuous cAMP signal within the mound (Schnitzler et al. 1995).

By activating expression of genes in cell culture assays in the absence of multicellular development, it is possible to identify essential regulatory molecules. The genes required for the multicellular stages of development can be divided into three major classes: postaggregative, prestalk, and prespore. Postaggregative genes are induced first within the developing mound and can be induced in

suspension cultures of cells starved for 6 hr in response to micromolar concentrations of extracellular cAMP in the absence of sustained cell–cell contact, contact with a substratum, or other signals (Mehdy and Firtel 1985; Schaap et al. 1986; Jermyn et al. 1987) (See Fig. 1). These genes include those required for morphogenesis and subsequent cell-type gene expression, such as the transcription factor GBF and the putative cell-surface regulatory protein LagC, genes whose expression becomes enriched in the prestalk cells (prestalk-enriched genes, e.g., *CP2* and *rasD*), and genes that are later preferentially expressed in ALCs [e.g., *Gα4* and *PTP1*] (Reymond et al. 1984; Pears et al. 1985; Datta et al. 1986; Hadwiger and Firtel 1992; Howard et al. 1992; Dynes et al. 1994). (See Tables 1 and 2 for the functions of these genes.) The prestalk (*ecmA* and *ecmB*) and prespore cell type-specific genes (Table 2) are induced ~2 hr after the postaggregative genes within the mound and require sustained cell–cell or cell–substrate contact in some strains as well as prior exposure to high levels of cAMP (see above; Kimmel and Firtel 1991; Williams 1991). The cell–cell contact may help maintain appropriate levels of diffusable molecules such as the morphogen DIF (differentiation-inducing factor, a chlorinated hexaphenone) or may involve specific signaling pathways mediated by cell-surface molecules (Dynes et al. 1994). The temporal pattern of induction of the postaggregative and cell type-specific genes in cell culture mimics the temporal pattern of induction during multicellular development (see Fig. 1). When cAMP is given to cells starved for 6 hr, the gene encoding the transcription factor GBF is the first to be induced. GBF transcripts are maximal after <1 hr of cAMP stimulation, whereas transcripts for *CP2* and *LagC* reach maximal levels after ~3–4 hr and expression of *ecmA* and the prespore gene *SP60/CotC* is not detected until 4 hr (Schnitzler et al. 1994, 1995). Removal of extracellular cAMP at any stage during induction results in a rapid deactivation of the genes (Mehdy et al. 1983; Hjorth et al. 1989).

It is the rise in extracellular cAMP in the mound, stimulating the activity of the transcription factor GBF, that is thought to activate postaggregative gene expression and initiate the transcriptional events leading to cell-type-specific gene expression (Figs. 4 and 5B). A conserved regulatory element, designated a G-box or CAE, has been identified in postaggregative and cell-type-specific gene promoters (Datta and Firtel 1988; Pears and Williams 1988; Hjorth et al. 1989, 1990; Pavlovic et al. 1989; Haberstroh and Firtel 1990; Haberstroh et al. 1991; Ceccarelli et al. 1992; Esch et al. 1992; Fosnaugh and Loomis, 1993; Powell-Coffman and Firtel 1994). These elements are required for gene expression during multicellular development and responsiveness to cAMP in cell suspension assays and bind a developmentally regulated DNA-binding protein designated GBF (Hjorth et al. 1989, 1990; Haberstroh and Firtel 1990; Haberstroh et al. 1991). In vivo and in vitro studies indicate that GBF directly mediates the expression of these genes. Mutations in either of two G-box/CAE half-sites eliminate the ability to bind GBF in vitro and for the site to function in

Figure 4. Regulation of GBF and postaggregative gene expression. GBF is expressed at low levels during growth, and the levels increase during early development. GBF expression and binding activity are induced to high levels as the mound forms and levels of intercellular cAMP increase or in response to exogenous cAMP, and they reach maximum levels very rapidly. Postaggregative genes, such as *LagC*, *CP2*, and *rasD*, are induced with a lag relative to GBF as GBF activity is required for their expression. In cells expressing GBF constitutively, postaggregative genes are rapidly induced with RNA being detected within 15 min. (See text for details and references.)

vivo. In addition, the affinity of the element for GBF directly corresponds with the level of induction of the gene in the mound or in response to cAMP. G-boxes/CAEs from prestalk-enriched and prespore-specific genes can be interchanged, indicating that the element does not confer cell-type specificity, only the ability to be expressed during multicellular development (Hjorth et al. 1990; Ceccarelli et al. 1992; Powell-Coffman 1994; Powell-Coffman et al. 1994). Cell-type-specific *cis*-acting regions have been identified for prestalk- and prespore-specific genes that function in association with G-boxes to mediate the correct pattern of cell-type expression (Datta and Firtel 1988; Ceccarelli et al. 1991, 1992; Haberstroh et al. 1991; Early et al. 1993; Powell-Coffman et al. 1994). GBF contains two centrally located, putative zinc fingers with homology to the zinc finger in the glucocorticoid receptor (Schnitzler et al. 1994).

gbf null cells have a particularly interesting phenotype. They aggregate normally and form a mound, but no postaggregative genes are induced, and development arrests (Fig. 5A; Schnitzler et al. 1994). Pulse-induced genes are induced and then repressed normally as the mound forms and cAMP levels rise, which presumably adapts the aggregation-stage signaling pathways. Thus, the repression of aggregation-stage events is mediated by the rise in cAMP in the mound and not the result of postaggregative gene expression. In this regard, the for-

mation of the mound functions as a developmental timer to induce postaggregative genes and repress aggregation-stage processes. The *gbf* null mutant thus effectively dissociates aggregation-stage and postaggregative stage development. The inhibition of pulse-induced genes in the mound can be mimicked in vitro by providing high, continuous cAMP to cells that have been induced previously by 30 nM cAMP pulses (Mann and Firtel 1989; Schnitzler et al. 1995). Interestingly, in *gbf* null cells, the newly formed mound then dissipates, presumably because it cannot undergo further morphogenesis and because the dynamic chemotactic pathways that originally formed the mound are repressed (Fig. 5A,B). As the mound dissipates, the pulse-induced genes are reinduced, presumably because of the decrease in extracellular cAMP levels as the mound falls apart and a resultant deadaptation of the aggregation-stage pathways. The cells then reaggregate to form a new mound, and the cycle repeats.

gbf null cells constitutively expressing GBF do not express the other postaggregative genes until formation of the mound, indicating that GBF is not sufficient to induce late genes (Schnitzler et al. 1994, 1995). However, when washed complemented *gbf* null vegetative cells are provided cAMP, the postaggregative genes are rapidly induced (Schnitzler et al. 1995). This indicates that a cAMP-mediated pathway, in addition to GBF, is required to induce late gene expression and that the signal transduction pathway necessary for inducing late genes is already present in vegetative cells. Why cannot high cAMP levels induce GBF and downstream genes in early wild-type cells? A type of built-in timing mechanism appears to prevent this. Wild-type vegetative cells have insufficient levels of GBF to activate this pathway. GBF expression is very low in vegetative cells and increases to a low level in response to starvation. When GBF levels are sufficient, GBF expression can be rapidly induced, either as the mound forms or in response to high cAMP levels (Fig. 4; Schnitzler et al. 1994, 1995). Consistent with this model, studies have suggested that GBF regulates its own expression, but definitive proof awaits analysis of the GBF promoter (Hjorth et al. 1989; Schnitzler et al. 1994). Also as predicted, the cell type-specific genes are not induced until cells enter the mound and respond to the high cAMP level, although they are competent to respond to cAMP in suspension culture several hours earlier. In cells constitutively expressing GBF, other postaggregative genes are induced very rapidly in response to cAMP, with levels of transcript reaching near maximal levels within 1 hr after stimulation. *ecmA* and *SP60* are also induced but only after several hours of exposure to cAMP, implying that there are genes downstream from GBF whose products are necessary for the induction of these genes (see below; Fig. 6).

Two independent signaling pathways regulate aggregation and postaggregative development

The above analysis suggests that two distinct signaling pathways, one responding to cAMP pulses and one to a higher, continuous level of cAMP, differentially regulate

Figure 5. Morphology and molecular phenotype of *gbf* null cells. (A) Developmental morphology of wild-type and *gbf* null cells. At 12 hr, *gbf* null cells have formed aggregates, and essentially all cells are in aggregates. At 20 hr, many of the cells have separated from the aggregates, which are flatter and more spread out. At 24 hr, they have reformed. The developmental expression of a pulse-induced gene (*D2*) is shown. In wild-type cells, its expression is induced during aggregation and repressed with mound formation. In *gbf* null cells, it is induced and repressed normally but then gets reinduced as the mounds have dissipated and re-repressed as the mounds reform. (B) A shift between cAMP pulses and continuous, high levels of cAMP within the aggregate mediates the developmental switch between aggregation and cellular differentiation. Cartoon of morphology and developmental signals in wild-type cells, *ga2* null cells with and without cAMP, and *gbf* null cells (see text for details). A shift between cAMP pulses and continuous, high levels of cAMP within the aggregate mediates the developmental switch between aggregation and cellular differentiation. *ga2* null cells will induce late genes if starved for 6 hr and then given high, continuous cAMP. If, after 12 hr, the cells are plated on agar, small fruiting bodies with mature spores are obtained. The fruiting bodies are abnormal, because of the absence of proper cAMP signaling in the absence of a $G\alpha$ -subunit to couple to the cARs (cAR4). This is also consistent with the abnormal culminants produced when a dominant-negative $G\alpha 2$ -subunit is expressed in prestalk cells.

aggregation and postaggregative development and cell-type differentiation. Earlier pharmacological studies suggested that the same cAMP receptors (or receptors with the same ligand-binding specificities) control aggregation and postaggregative development (Schaap and Van Driel 1985; Gomer et al. 1986a; Haribabu and Dottin 1986; Oyama and Blumberg 1986). The differences between the aggregation-stage and postaggregative pathways were uncovered when the ability of cAMP to induce postaggregative genes was examined in various mutant strains constitutively expressing GBF (Schnitzler et al. 1995). Studies using deletion mutants of cAR1 and cAR3 showed that either is essential, indicating that the same receptors regulate aggregation and postaggregative gene expression. Unexpectedly, postaggregative genes

are induced normally in cells expressing GBF but lacking either $G\alpha 2$, which couples to cARs, or the sole known $G\beta$ -subunit, both of which are required for aggregation (Schnitzler et al. 1995). Studies of the biochemical properties of the *gβ* null cells strongly suggest that there is only a single $G\beta$ subunit in *Dictyostelium* (Wu et al. 1995), although one cannot exclude a $G\beta$ subunit that is expressed at very low levels or has unusual biochemical properties. This suggests that cAMP receptor activation of postaggregative gene expression mediated by GBF functions through a signaling cascade that does not require a coupled G protein, a previously undefined pathway by which seven transmembrane receptors might signal. This pathway is outlined in Figure 2. Although G proteins do not appear to be required for receptor activa-

Figure 6. Model of signaling to control cell-type differentiation. (A) Model of the signaling pathways. A low level of GBF is expressed during starvation. As the mound forms, cAMP levels rise, which activates GBF and downstream gene expression (postaggregative genes) through a cAMP receptor-mediated signaling pathway that may involve the post-translational modification of GBF or an associated protein. This induces the postaggregative genes, including *LagC*, which is essential for cell-type-specific gene expression. *LagC* may be involved in cell-cell contacts that promote prestalk/prespore gene expression. It may feed back to enhance GBF function and the expression of other primary genes, facilitating the activation of prestalk and prespore genes. DIF is required for the prestalk pathway and inhibits the prespore pathway. GSK-3 is required for prespore gene expression. In *gsk-3* null cells, essentially all cells differentiate into prestalk cells. "Folate" refers to the requirement of Gα4, which couples to folate/pterin receptors and is required for prespore/spore differentiation. (B) DIF is required both for prestalk A/O (*ecmA*) and prestalk B (*ecmB*) induction. cAMP enhances *ecmA* and represses *ecmB*. Models of cAMP oscillations within the slug suggest that the prestalk B cells are localized in a region of low cAMP levels. The geometry of the oscillator may control the spatial localization of prestalk A/O and prestalk B cells within the anterior region. (See text for details and references.)

tion of the postaggregative genes such as *LagC*, *rasD*, and *CP2*, Gβ is required for the subsequent induction of the cell-type-specific genes, consistent with the observation that the level of prespore and prestalk gene expression is reduced in *gα4* null cells (Hadwiger and Firtel 1992; Schnitzler et al. 1995). Other studies show that cAMP also activates a Ca^{2+} influx by a pathway that, by the same criteria, is G protein-independent (Milne et al. 1995).

The independence of the pathways controlling aggregation and cell-type differentiation is highlighted by several studies. As described above, Gα2 is required for mediating signal transduction events essential for in vivo aggregation, and no other Gα subunit has been found to substitute for Gα2 (Wu et al. 1994a,b). When 6-hr starved *gα2* null cells (not expressing GBF constitutively) are given high levels of cAMP, postaggregative and cell-type-specific genes are induced with kinetics similar to wild-type cells (Schnitzler et al. 1995). When these cells are then placed on agar, small agglomerates form that differentiate into fruiting bodies (Fig. 5B). Although the morphology is not fully wild-type, mature spores are formed. Consistent with the induction of postaggregative development being Gα2 independent, expression of a dominant-negative form of Gα2 blocks cAMP-mediated responses when expressed during aggregation but has no effect on cell-type differentiation when expressed from either a prestalk- or prespore-specific promoter, although expression from the prestalk promoter does lead to abnormalities in stalk morphogenesis during culmination (Carrel et al. 1994).

The identification of potentially G protein-independent pathways regulated by seven-span receptors is novel, although evidence for such pathways in other systems does exist. A G protein-independent response has been reported for yeast in response to pheromone (Jackson et al. 1991). In mammalian cells, the binding and

phosphorylation of both the β-adrenergic receptor and rhodopsin by their respective kinases requires activation by ligand binding or light, respectively, but is independent of heterotrimeric G proteins (Palczewski et al. 1991; Chen et al. 1993). In addition, it has been suggested that the angiotensin II receptor may function via a G protein-independent pathway (Kambayashi et al. 1993; Mukoyama et al. 1993), although no such definitive pathways have been identified. We expect that more extensive G protein-independent pathways exist in higher organisms but may not have been identified because of the inability to use extensive genetic manipulation or the absence of the necessary assays. The recent identification of previously unknown seven-span receptor-mediated pathways in mammalian cells, such as the activation of PI3 kinase (Stephens et al. 1994), suggests that the identification of all pathways may be far from complete.

Requirements for cell-type-specific gene expression: cell-cell interactions and the DIF morphogen

In addition to high cAMP levels, cell-cell interactions or cell-substrate interactions and products of the postaggregative genes are essential to induce the prestalk- and prespore-specific late genes, as described above. One gene required for cell-type-specific gene expression is *LagC*, which encodes a putative cell-surface signaling protein with a predicted single transmembrane domain and a long extracellular domain (Dynes et al. 1994). *LagC* lies downstream from GBF and requires both GBF and cAMP for expression. Like *gbf* null cells, *lagC* null cells form mounds that dissipate and re-form with development arresting at this stage. *lagC* null cells express the other primary postaggregative genes (*CP2* and *rasD*) in vivo or in response to cAMP (although at low levels) but not the cell-type-specific genes. *LagC* functions cell

nonautonomously, as *lagC* null/wild-type cell chimeras develop normally and cell-type-specific genes are induced in the *lagC* null cells. The putative structure of LagC as a cell-surface protein is consistent with its proposed function as a signaling molecule in cell-cell interactions. These results suggest that cAMP activates GBF expression, which then induces the expression of LagC, which in turn mediates a process that is required for cell-type-specific gene expression and cell-type differentiation. LagC does not appear to function as a cell adhesion molecule *per se* because overexpression of LagC does not cause apparent increased cell adhesion. Although the mechanism by which LagC functions is not known, it may function, in part, as a feedback stimulator of GBF expression, which is lower in *lagC* null cells than in wild-type cells (Fig. 6).

The morphogen DIF induces single cells to differentiate into stalk cells in monolayer culture and is essential for the induction of *ecmA* and *ecmB* and for prestalk/stalk cell differentiation (Town et al. 1976; Berks et al. 1991; Williams 1991). Extracellular cAMP is necessary to achieve sufficient developmental competence for prestalk cell differentiation through the GBF pathway. However, late in the process of cell-type differentiation, DIF and cAMP are antagonists in the expression of *ecmB* (DIF induces; cAMP represses). For *ecmA*, cAMP stimulates DIF-dependent expression. For prespore genes, cAMP induces expression, whereas DIF inhibits expression (Early and Williams 1988). The mechanism by which DIF regulates gene expression is not known; however, a cytosolic binding protein has been identified (Insall and Kay 1990).

Among the unsolved questions are, How do cells respond differentially to the DIF present in the developing mound? and What is the mechanism by which DIF functions? In the whole organism, DIF induces only a small percentage of cells to become prestalk cells. Thus, how do the remaining cells differentiate into prespore cells in the presence of DIF? It is possible that cells with a propensity to become prestalk cells (cells starved earlier in the cell cycle) are more sensitive to DIF and may induce a positive feedback pathway that increases their sensitivity to reduced levels of DIF. Prestalk B cells, which require DIF for their induction, have been reported to synthesize high levels of DIF *in vitro* (Kwong et al. 1990). In addition, the developing prestalk cells may act as a sink for the morphogen, reducing the overall concentration within the mound (Insall et al. 1992; Kay et al. 1993). Consistent with this idea, prestalk cells have a higher concentration of the enzymes required to metabolize and inactivate DIF than prespore cells. These enzymes are induced by DIF, providing a feedback loop (Insall et al. 1992). In the slug, DIF is present in the posterior at a higher concentration than in the tip, possibly as a result of a higher rate of metabolism in the prestalk cells (Brookman et al. 1987). The function of DIF in the prespore zone, if any, is unknown. It may be involved in maintaining the differentiated state of ALCs and/or in modulating prespore gene expression (see below).

GSK-3, PKA, and other regulatory molecules controlling cell fate

What other components are required for cell-type differentiation? What components regulate the prestalk/pre-spore ratio? It is now clear that these pathways are complex and involve PKA and GSK-3, which also control cell-fate decisions in *Drosophila*. In *Drosophila*, GSK-3, the product of the *shaggy/zest-white 3* locus, is required for multiple cell-fate decisions during embryogenesis and is thought to function downstream from *wingless* (Siegfried et al. 1990, 1992; Simpson et al. 1993). In *Dictyostelium*, GSK-3 plays a crucial role in the decision-making process between prestalk and prespore cell pathways (see model in Fig. 6). GSK-3 is required for prespore differentiation as *gsk-3* null cells differentiate to yield a high percentage of *ecmB*-expressing prestalk cells, whereas prespore cell differentiation is minimal (Harwood et al. 1995).

GSK-3 regulation of prespore/prestalk cell-fate determination may involve pathways controlled through the cAMP receptors cAR2 and cAR4. In wild-type cells, extracellular cAMP inhibits differentiation of the prestalk B cells once the pathways are initiated by the induction of GBF. However, this does not occur in *gsk-3* null cells. This, plus the lack of prespore gene expression in *gsk-3* null cells, suggests that GSK-3 functions to activate the prespore pathway and repress the prestalk B pathway in response to cAMP. This phenotype is the opposite of that seen in *car2* or *car4* null cells (Saxe et al. 1993; Louis et al. 1994). This suggests that cAR2 and cAR4, which are preferentially expressed in prestalk cells, may function to inhibit GSK-3 function and suppress prespore differentiation. cAR2 and cAR4 may function through the production of a diffusible substance, produced in the anterior, prestalk region that would act to repress prespore gene expression and help establish the prestalk/prespore boundary by negatively regulating GSK-3 function (Harwood et al. 1995). Alternatively, cAR2 and cAR4 may negatively regulate GSK-3 via a cell-autonomous pathway within prestalk cells. The spatial pattern of GSK-3 expression is not yet known; however, because it is expressed throughout growth and development, it may be present in all cells.

The heterotrimeric G protein α -subunit G α 1 (see below) and PKA are also involved in prestalk AB cell differentiation. PKA, which has been shown recently to be important in cell-fate determination in *Drosophila* (Perimon 1995), is thought to regulate a repressor element in the *ecmB* promoter (Ceccarelli et al. 1991; Harwood et al. 1993). Removal or mutation of this element expands *ecmB* expression into the entire anterior prestalk region. The ability of the repressor to inhibit *ecmB* expression in prestalk A cells is negatively regulated by PKA (Harwood et al. 1993; Williams et al. 1993a). In *gsk-3* null cells, the inhibition of *ecmB* expression by extracellular cAMP is lacking, suggesting that GSK-3 and PKA may be a component of the same pathway. Interestingly, PKAc α t expression is high in the very anterior of the slug in prestalk A and AB cells (Mann et al. 1994). PKA is also

Firtel

required cell autonomously for the initial induction of both the prestalk and prespore pathways (Mann and Firtel 1991; Williams et al. 1993b). The heterotrimeric $G\alpha$ subunit $G\alpha 1$ appears to function as a negative regulator of prestalk AB differentiation and morphogenesis. $G\alpha 1$ and *ecmB* have indistinguishable patterns of expression, and expression of a dominant activating $G\alpha 1$ subunit from the $G\alpha 1$ promoter results in a loss of *ecmB* expression in the anterior core of prestalk AB cells and abnormal slug morphogenesis and culmination (Dharmawardhane et al. 1994).

Increases in intracellular Ca^{2+} have been implicated in the induction and maintenance of the prestalk pathway. Prestalk cells are known to sequester Ca^{2+} and have higher cytosolic levels of Ca^{2+} (Maeda and Maeda 1973; Tirilapur et al. 1991; Schlatterer et al. 1992; Cubitt et al. 1995). Artificially increasing the cytosolic Ca^{2+} levels using thapsigargin and Ca^{2+} ionophore causes a significant increase in the percentage of cells that finally differentiate into stalk cells, at the expense of spore cells (Kubohara and Okamoto 1994). Although modulation of the level of Ca^{2+} appears to affect the ratio of prestalk and prespore cells, it is unclear whether Ca^{2+} functions as a "determinant" to promote some cells to become prestalk cells. Other gene products required for prestalk or prespore cell-type differentiation are being identified in a number of laboratories using REMI insertional mutagenesis; thus, the complexity of these pathways is expected to increase significantly in the near future.

ALCs serve as essential regulatory components

ALCs fate map to the lower cup of the sorus and the basal disc, as opposed to the anterior prestalk cells, which predominantly form the stalk (Jermyn and Williams 1991). When posterior, prespore regions are dissected from the whole slug, the ALCs form a new tip and new ALCs are recruited from the prespore population (Sternfeld and David 1982). Recent studies suggest that these cells also have a regulatory role within the slug. A number of regulatory gene products have been shown to be preferentially expressed in ALCs during the multicellular stages, with little or no expression in prestalk or prespore cells. These include the MAP kinase ERK1, two protein-tyrosine phosphatases (PTP1 and PTP2), and $G\alpha 4$ (Howard et al. 1992, 1994; Gaskins et al. 1994; Hadwiger et al. 1994).

The strongest evidence for a cell nonautonomous role of ALCs in controlling developmental decisions is provided by studies on the heterotrimeric $G\alpha$ subunit $G\alpha 4$, whose expression during the multicellular stages requires induction by cAMP. $g\alpha 4$ null cells arrest at the first finger stage and express prespore genes at very low levels (Hadwiger and Firtel 1992). Furthermore, spore production in $g\alpha 4$ null cells is reduced approximately four orders of magnitude. When these cells are mixed with wild-type cells, normal-appearing fruiting bodies are formed and there is a partial complementation of the spore production defect in the $g\alpha 4$ null cells. These and other studies suggest that $G\alpha 4$ functions in ALCs to reg-

ulate differentiation of adjacent prespore cells. $G\alpha 4$ does not couple to cAMP receptors but couples to at least two classes of receptors that bind folate and other pterins [folic acid receptors (FARs)] (Hadwiger et al. 1994), although $G\alpha 4$ may also couple to a receptor for an unknown ligand. These results suggest that this or another pterin functions as an essential signaling molecule during the multicellular stages. *Dictyostelium* cells produce a pterin with a structure similar to a pterin that can activate $G\alpha 4$ -dependent pathways during the multicellular stages, which may be the molecule mediating $G\alpha 4$ pathways (Klein et al. 1990; Hadwiger et al. 1994).

Culmination—regulation by PKA and other signaling pathways

Slugs migrate phototactically to directional light until the environment is optimal for fruiting body formation (Loomis 1975). During this time, the spatial patterning of the slug remains constant and little differentiation into stalk and spores takes place. High NH_4^+ and humidity in the absence of overhead light inhibits culmination. Viewed teleologically, this allows the slug to migrate from beneath decaying leaves on the forest floor before culminating to optimize dispersion of the spores. Under laboratory conditions, migrating slugs stop immediately when exposed to overhead light and initiate culmination. Prestalk A cells enter a cone formed at the top of the stalk tube, induce a stalk cell-specific component of the *ecmB* promoter, and differentiate into stalk cells (Williams et al. 1993b). The forming stalk tube elongates through the prespore mass, lifting it off the substratum. Simultaneously, the ALC population migrates to form the basal disc and the upper and lower cups of the maturing sorus. The analysis of mutants that affect $G\alpha 2$, cAR4, and PDE all point to the morphogenetic movements being controlled by extracellular cAMP and receptor/ $G\alpha 2$ -mediated pathways (Okaichi et al. 1992; Hall et al. 1993; Louis et al. 1994).

Midway through formation of the stalk tube, prespore cells begin to differentiate into spore cells. *spiA* is not expressed until prespore cells begin terminal differentiation and is a marker for spore cell differentiation (Richardson et al. 1991). *spiA/lacZ* staining initiates at the top of the sorus and proceeds toward the base, indicating a gradient of prespore $\rightarrow$ spore differentiation (Richardson et al. 1994). Several findings suggest that PKA activation triggers this induction. First, strains expressing a dominant-negative PKA-R subunit from a prespore promoter produce fruiting bodies that contain undifferentiated amoebae in the sorus (Hopper et al. 1993b). Second, strains that lack a functional PKA-R subunit or express PKAcat from a prespore promoter develop rapidly and form spores precociously (Simon et al. 1992; Mann and Firtel 1993; Mann et al. 1994). Finally, *spiA* expression and spore cell differentiation can be induced in disaggregated slug cells by 8-Br-cAMP (Kay 1989; Riley et al. 1989; Richardson et al. 1991, 1994). In addition, similar experiments suggest that PKA regulates stalk cell differ-

entiation independently (Harwood et al. 1992; Hopper et al. 1993a; Williams et al. 1993a).

The *in vivo* mechanism of PKA activation is not known. One might assume the activation is through an increase in intracellular cAMP, which is known to accompany culmination. However, cells lacking the *ACA* gene, and thus in theory unable to make cAMP, can be coaxed into making abnormal fruiting bodies with spores if given cAMP analogs that are specific for cell-surface receptors (Pitt et al. 1993). Whereas the increase in PKA activity may be regulated by increases in intracellular cAMP, the results in the *aca* null cells suggest that other pathways may be used *in vivo*. Ammonia, which blocks culmination, may inhibit PKA activation, possibly by blocking the increase in intracellular cAMP, or it may inhibit a downstream pathway (Wang and Schaap 1989; Davies et al. 1993; Hopper et al. 1993b; Gee et al. 1994). Recent evidence suggests that NH_4^+ may regulate the pH of a cytosolic vacuolar compartment, which has been proposed to control, directly or indirectly, the activation of AC (Gross et al. 1983, 1988; Davies et al. 1993). In the maturing fruiting body, the *PKAcat* gene is preferentially expressed in the maturing spore cells, perhaps to a level that oversaturates the PKA regulatory subunits (Mann et al. 1994).

Biochemical analysis has identified a rapid rise in the potential second messenger 1,2-diacylglycerol (DG) that occurs at the onset of culmination, and elevated DG levels are maintained throughout the time of stalk cell differentiation (Cubitt et al. 1993). This increase in DG, which probably results from the activation of PLD and not PLC, may be involved in regulating the induction of culmination. DG levels are induced to maximal levels within 2 min in slugs in response to overhead light, which induces culmination. The extent of the increase in DG levels depends on the level of $\text{G}\alpha 1$, which plays an important role in culmination (Dharmawardhane et al. 1994). Mutants (sluggers) that show extended slug migration and delayed culmination are hypersensitive to NH_4^+ (Gee et al. 1994), as are strains expressing the dominant-negative PKA-R from a prespore promoter. It is possible that NH_4^+ could be affecting multiple pathways, including the PKA pathway and that leading to DG production.

Developmental themes, insights, and the future

The analysis of signaling pathways in *Dictyostelium* has led to new insights into how organisms solve common developmental problems and an understanding of the underlying molecular mechanisms. The *Dictyostelium* developmental program, like that of metazoans, is separated into two major phases: the formation of the multicellular organism and cell-type differentiation and morphogenesis. Whereas metazoans form a multicellular organism through the division of the zygote, the multicellular organism in *Dictyostelium* is formed through the coaggregation of independent cells, using G protein-coupled signaling pathways that are similar to those used to mediate chemotaxis in neutrophils and that in-

volve both the activation of downstream effectors and a requisite period of adaptation. The analysis of the pathways leading to aggregation and the developmental switch between the formation of the multicellular organism and its subsequent differentiation has yielded new concepts about how receptors perceive ligands: The pulsatile signal that controls aggregation and the continuous cAMP signal that controls multicellular differentiation activate distinct signaling pathways from the same receptors. Although many of the pathways leading to aggregation are regulated through classic G protein-coupled pathways, the activation of GBF function is mediated through distinct pathways that may be G protein-independent. Developmental timing is solved by making use of these cAMP-mediated pathways. During aggregation, cells have the ability to respond to either a pulsatile or continuous signal. The formation of the mound, which results in an elevation of the levels of cAMP, initiates the developmental switch in signaling pathways by repressing aggregation-stage pathways and activating GBF function. Not unexpectedly, downstream cell-type differentiation uses cell-cell interactions and signaling molecules such as GSK-3 and PKA, now known to regulate cell-fate decisions in metazoans. The more complex patterning of the multicellular organism and the orchestration of cell movements are mediated through a series of other signaling pathways, some of which use heterotrimeric G proteins as key regulatory molecules. Others use morphogens such as DIF and NH_4^+ which most likely function through yet other molecular mechanisms.

In *Dictyostelium*, many of the developmental decisions are controlled by seven-span/serpentine receptors that couple to heterotrimeric G protein-dependent, and possibly independent, pathways. In metazoans, significant attention has been given to both tyrosine kinase and transforming growth factor- β (TGF β) cell-surface receptors as regulators of cell-fate decisions (Jessell and Melton 1992; Van der Geer et al. 1994). The importance of tyrosine phosphorylation in *Dictyostelium* is highlighted by the developmental function of at least three spatially and temporally regulated protein-tyrosine phosphatases (Howard et al. 1992, 1994) that are known to play an important role in various stages of *Dictyostelium* development as well as in controlling responses of starved cells to growth stimulation (Howard et al. 1993). Two tyrosine kinases have been identified in *Dictyostelium*, but these are probably not cell-surface receptors (Tan and Spudich 1990).

In both *Dictyostelium* and metazoans, the specificity of the signaling pathways is controlled by a combination of the spatial/temporal expression of both receptors and ligands plus the coupled intracellular signaling pathways. The emphasis on how G protein-coupled pathways control development in *Dictyostelium* may be a function of early studies showing the importance of cAMP-regulated pathways, which led to the identification and cloning of genes encoding cAMP receptors and eight distinct $\text{G}\alpha$ subunits. It is expected that REMI insertional mutagenesis will identify other receptors and signaling

Firtel

pathways, including those regulated by tyrosine phosphorylation. It is also anticipated that further analysis of G protein-coupled pathways in metazoans will uncover their importance in controlling developmental events. The presence in *Dictyostelium* of at least two MAP kinases with specific developmental functions (Gaskins et al. 1994; Segall et al. 1995) suggests that additional signaling pathways remain to be elucidated.

The ability to dissect pathways using a combination of genetic, molecular, and biochemical approaches in *Dictyostelium* should allow rapid progress in coupling these mutants into defined biochemical pathways that regulate development in this system. Moreover, this should result in new insights into the control of signaling pathways common to all eukaryotes. The history of the analysis of yeast, *Drosophila*, and *Caenorhabditis elegans* indicates that much can be learned about basic cellular decisions by studying a variety of systems that have powerful genetic, molecular, and biochemical tools. Examination of the cytoskeleton and myosin function in *Dictyostelium* has led to major insights into the mechanisms by which molecular motors work and into the role of the cytoskeleton in all eukaryotes. The insights into G protein-coupled pathways and activation of AC and chemotaxis, the identification of a molecular function for GSK-3, and the understanding of receptor activation of GBF should be directly applicable to all eukaryotes. The rapidity with which gene function can be examined in *Dictyostelium* should facilitate our understanding of many of the presently described pathways as well as identify pathways that may pioneer studies in other systems.

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Integration of signaling information in controlling cell-fate decisions in Dictyostelium.

R A Firtel

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