

REVIEW

Regulation of Cell-Fate Determination in *Dictyostelium*

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A key step in the development of all multicellular organisms is the differentiation of specialized cell types. The eukaryotic microorganism *Dictyostelium discoideum* provides a unique experimental system for studying cell-type determination and spatial patterning in a developing multicellular organism. Unlike metazoans, which become multicellular by undergoing many rounds of cell division after fertilization of an egg, the social amoeba *Dictyostelium* achieves multicellularity by the aggregation of $\sim 10^5$ cells in response to nutrient depletion. Following aggregation, cell-type differentiation and morphogenesis result in a multicellular organism with only a few cell types that exhibit a defined patterning along the anterior–posterior axis of the organism. Analysis of the mechanisms that control these processes is facilitated by the relative simplicity of *Dictyostelium* development and the availability of molecular, genetic, and cell biological tools. Interestingly, analysis has shown that many molecules that play integral roles in the development of higher eukaryotes, such as PKA, STATs, and GSK-3, are also essential for cell-type differentiation and patterning in *Dictyostelium*. The role of these and other signaling pathways in the induction, maintenance, and patterning of cell types during *Dictyostelium* development is discussed. © 1999 Academic Press

Key Words: *Dictyostelium*; signaling pathways; cAMP; receptors; cell fate.

INTRODUCTION

The basic element of all multicellular development is the initial divergence of specialized cell types to generate functional tissues. In metazoans, which become multicellular by division of a zygote, both asymmetric cell division and intercellular communication provide information that directs specialization of cell types (Bowerman, 1998; Davidson *et al.*, 1998; Dierick and Bejsovec, 1999; Sundaram and Han, 1996). *Dictyostelium* is unicellular under optimal growth conditions. Nutrient depletion triggers multicellular development, which culminates in the production of a fruiting body consisting of a mass of dormant spores held aloft by a rigid stalk constructed of vacuolated cells (Aubry and Firtel, 1999; Firtel, 1995, 1996; Ginsburg *et al.*, 1995; Loomis and Cann, 1982; Parent and Devreotes, 1996; Williams, 1995). This developmental program is notable for its

relative simplicity. The entire process takes ~ 24 h and involves the generation of only a few distinct cell types. However, the pathways required for the specification of divergent cell types depend on factors similar to those in metazoans: preexisting information contained within each cell and intercellular communication. *Dictyostelium* development requires highly conserved molecules, such as STATs, PKA, and GSK-3, which are also important for metazoan development (Harwood *et al.*, 1995; Kawata *et al.*, 1997; Kay, 1997; Mann and Firtel, 1991; Mohanty *et al.*, 1999; Simon *et al.*, 1992). Mutational analysis, facilitated greatly in recent years by the use of gene knockouts, insertional mutagenesis, and shotgun antisense technology has allowed an in-depth study of cell-type induction and patterning in *Dictyostelium* (Kuspa and Loomis, 1992; Mann *et al.*, 1994a; Gomer, 1998, 1999). In addition, examination of intercellular signaling by mosaic analysis is easily performed by mixing strains together and allowing them to codevelop as a chimera (Dynes *et al.*, 1994). Available EST databases and the ongoing sequencing of the *Dictyostelium* genome have revealed further similarities between the

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genetic control of *Dictyostelium* multicellular development and that of higher eukaryotes (http://dicty.cmb.nwu.edu/dicty/dictyostelium_genomics.htm) (Kay and Williams, 1999; Morio *et al.*, 1998).

The formation of a multicellular organism in *Dictyostelium* results from the chemotactic aggregation of up to 10^5 cells (Firtel, 1995; Loomis, 1996; Loomis *et al.*, 1998; Williams, 1995). Accumulation of secreted density-sensing factors regulates the initial expression of gene products required for aggregation (Clarke *et al.*, 1987; Gomer *et al.*, 1991; Jain *et al.*, 1992, 1997; Klein and Darmon, 1976; Mehdy and Firtel, 1985; Rath *et al.*, 1991; Brock and Gomer, 1999). Initial production of cAMP by any given cell causes neighboring cells to rapidly induce the activation of adenylyl cyclase and release cAMP into the extracellular medium. Chemoattractant stimulation simultaneously causes a temporary decrease in cAMP-receptor affinity and adaptation of the signaling pathways, leading to the unidirectional outward relay of the cAMP signal and an auto-regulatory loop that is required for the high-level expression of many of the genes involved in these signaling pathways (Devreotes, 1994; Dinauer *et al.*, 1980; Kesbeke *et al.*, 1985). Degradation of cAMP by both intracellular and extracellular phosphodiesterases leads to resensitization of cAMP-stimulated pathways (Franke and Kessin, 1992; Shaulsky *et al.*, 1998; Thomason *et al.*, 1998), thereby permitting the relay of another wave of chemoattractant. *Dictyostelium* cells are exquisitely sensitive to cAMP gradients and undergo chemotaxis toward the source of the signal (Aubry and Firtel, 1999; Parent *et al.*, 1998). Many of the components of cAMP relay and chemotaxis have been identified and studied, including classical signal transduction molecules and novel proteins; however, space does not permit a thorough discussion of these processes.

Directed chemotaxis toward cAMP results in the production of a hemispherical mound. Subsequently, an apical tip is formed that begins to extend upward. This tip elongates into a finger-shaped structure that falls onto the substratum to yield a "slug" that can migrate toward light, heat, or various chemicals, a behavior that, in the natural habitat, leads to deposition of the fruiting body in an more advantageous environment for spore dispersal (reviewed in Fisher *et al.*, 1984).

Spatial organization of specialized cells is most apparent at the slug stage when distinct cell types are arranged along the anterior-posterior axis (Fig. 1). Cells that will ultimately differentiate into spores (prespore cells) are found in the posterior 80% of the slug. Cells that will eventually make up the stalk (prestalk cells) are localized to the anterior tip and constitute about 20% of the slug. Fusion of *lacZ* to the promoter region of *ecmA*, which encodes an extracellular matrix protein, has been used to subdivide the prestalk population into several classes: (i) prestalk A cells (pstA), which are visualized using a distal *ecmA* promoter/*lacZ* fusion (designated *ecmA/lacZ*), are found in the anteriormost 10% of the slug; (ii) prestalk O cells (pstO), which stain with a proximal *ecmA* promoter region/*lacZ* fusion

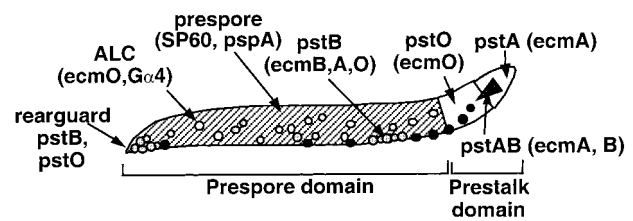


FIG. 1. Cell-type distribution in the *Dictyostelium* slug. Cell types are identified by the expression of promoter/*lacZ* fusions. The entire anterior prestalk domain, along with the ALCs, can be visualized using a *lacZ* fusion to the whole *ecmA* promoter (termed *ecmA/lacZ*). Deletion fragments of the *ecmA* promoter allow identification of prestalk subtypes. A distal promoter fusion (*ecmA/lacZ*) is expressed in pstA cells, whereas a proximal promoter fusion (*ecmO/lacZ*) is expressed in pstO cells and ALCs. PstB cells are defined by their high-level expression of *ecmB*, although some *ecmA/lacZ* and *ecmO/lacZ* staining is observed. See text for references.

(designated *ecmO/lacZ*), form a domain between the pstA cells and the prespore zone; and (iii) prestalk B cells (pstB), which express another extracellular matrix protein, *ecmB*, are found as a small population at the extreme posterior designated the "rearguard" and as a band of cells at the base of the slug near the prestalk-prespore boundary (Dormann *et al.*, 1996; Jermyn *et al.*, 1996). A cone of cells at the extreme anterior expresses both *ecmA* and *ecmB* and these cells are referred to as pstAB cells (Ceccarelli *et al.*, 1991; Dingermann *et al.*, 1989; Early *et al.*, 1993; Fosnaugh and Loomis, 1993; Gomer *et al.*, 1986; Haberstroh and Firtel, 1990; Jermyn *et al.*, 1989; Jermyn and Williams, 1991; Sternfeld, 1992). A fourth class of cells, anterior-like cells (ALCs), is found dispersed throughout the slug and displays many of the same characteristics as prestalk cells (Devine and Loomis, 1985; Firtel, 1995; Gaskell *et al.*, 1992; Loomis, 1982; Sternfeld and David, 1982). The ALC population constitutes ~10% of the prespore compartment and contains overlapping subsets of cells expressing *ecmA* and *ecmB*, as well as other markers not highly expressed in the anterior prestalk population. ALCs play an important role in the regulation and maintenance of cell-type proportions (Abe *et al.*, 1994; Mann and Firtel, 1993; Mann *et al.*, 1994b; Sternfeld and David, 1981). Accordingly, the expression of some genes encoding regulatory proteins are either highly enriched or specific for ALCs (Esch and Firtel, 1991; Gaskins *et al.*, 1994; Hadwiger and Firtel, 1992; Hadwiger *et al.*, 1996; Howard *et al.*, 1992). ALCs ultimately form the upper and lower cups that surround the spore mass. The pstAB and pstB cells and ALCs combine to form the basal disc, the structure that attaches the fruiting body to the substratum (Jermyn *et al.*, 1996; Sternfeld, 1992).

Prestalk and prespore cells are first visible as the mound forms toward the end of aggregation. Careful examination shows that, although the pstO population initially displays no defined pattern, the pstA cells are localized to the outer

edge of the aggregate (Early *et al.*, 1995). The pstA cells preferentially sort to the apex of the mound and are flanked by the pstO cells (Early *et al.*, 1993). pstB cells first appear throughout the aggregate before sorting to the bottom of the mound as it forms (Jermyn *et al.*, 1996; Williams *et al.*, 1989). The mechanism utilized for sorting of the cell types remains unclear, but it probably involves differential chemotaxis toward cAMP and possibly selective cell adhesion (Ginger *et al.*, 1998; Levine *et al.*, 1997; Siegert and Weijer, 1992, 1995; Sternfeld, 1979) (A. Nicol, W.-J. Rappel, H. Levine, and W. F. Loomis, submitted for publication). pstA cells chemotax more rapidly than other cell types toward cAMP and, along with the pstO cells, move from the apex to the base if a mound is placed on agar containing cAMP (Early *et al.*, 1995; Mee *et al.*, 1986; Traynor *et al.*, 1992). The requirement for cAMP in cell-type sorting was called into question by the demonstration that overexpression of the protein kinase A catalytic subunit (PKA-C) in *aca* null cells, which lack the major adenylyl cyclase expressed during aggregation, can induce the individual cell types and form properly proportioned fruiting bodies (Wang and Kuspa, 1997). However, another adenylyl cyclase activity, ACB, which is observed during multicellular development, has recently been discovered (Kim *et al.*, 1998; Meima and Schaap, 1999). ACB is likely to be encoded by a newly identified adenylyl cyclase gene, *AcrA* (Soderbom *et al.*, 1999). In *acrA* null cells, an adenylyl cyclase activity with a developmental profile similar to that described for ACB is absent. *AcrA* has some characteristics of a two-component system response regulator, suggesting it may be regulated by a two-component histidine kinase. It is possible that in *aca null/PKA-C* overexpressing cells, extracellular cAMP is supplied by ACB activity. If this is the case, it would support earlier models of the essential role of cAMP receptor-mediated signaling in cell sorting. Alternatively, *AcrA* protein may have functions during development in addition to being an adenylyl cyclase.

CELL-TYPE INDUCTION

One of the well-known hallmarks of *Dictyostelium* development is the extreme plasticity of cell-type differentiation. Early experiments by Raper demonstrated that, if separated from each other, the anterior prestalk and posterior prespore portions of a slug can regenerate the missing cell types to yield a normal fruiting body in the absence of cell division (Raper, 1940; Sakai, 1973). This indicates the presence of organism-wide homeostasis mechanisms that maintain the correct ratio of cell types. However, such studies give no clues as to how cell-type proportions are established or maintained. Raper's slug-cutting experiments demonstrate that each cell continues to retain the potential to become either prestalk or prespore, suggesting that cell fate is regulated by position-dependent morphogen signals within a developing organism. Indeed, cell-type choice can be regulated *in vitro* through the combinatorial

control of exogenous cAMP and DIF-1, a membrane-permeable chlorinated hexanophenone secreted by developing cells (Berks and Kay, 1990; Berks *et al.*, 1991; Kay, 1998; Town *et al.*, 1976; Williams, 1991). In addition, a mutant strain (HM44) which makes little or no DIF-1 is unable to induce prestalk genes (Kopachik *et al.*, 1983). Treatment of HM44 with exogenous DIF-1 rescues prestalk gene expression, supporting a role for DIF-1 in prestalk cell differentiation. Extracellular cAMP is detected by a family of four cell-surface serpentine cAMP receptors (cAR1-4) that are each expressed in a spatially and temporally specific manner and show different affinities to cAMP (Johnson *et al.*, 1993; Louis *et al.*, 1993, 1994; Rogers *et al.*, 1997; Saxe *et al.*, 1991, 1993). Induction of all cell-type-specific genes requires initial exposure to cAMP, which directs the expression of genes that regulate the early stages of multicellular development. Subsequent treatment with micromolar concentrations of cAMP alone leads to expression of the prespore genes *pspA* and *SP60/cotC*, whereas simultaneous application of DIF-1 represses prespore gene expression and induces the prestalk-specific gene *ecmA* (Berks and Kay, 1990; Fosnaugh and Loomis, 1991; Jermyn *et al.*, 1987; Pears and Williams, 1988). *ecmB* is also induced by DIF-1 after prior treatment with cAMP, but unlike *ecmA*, is repressed by the continued presence of cAMP (Berks and Kay, 1990). However, it is not clear how these findings relate to the role of DIF-1 in the differentiation of cell types in developing organisms. At the slug stage, the anterior contains higher concentrations of cAMP, whereas DIF-1 levels are unexpectedly higher in the prespore zone (Brenner, 1977; Brookman *et al.*, 1987; Kay *et al.*, 1993). In addition, DIF-1 rapidly induces the expression of the degradative enzyme DIF-1 dechlorinase (Insall *et al.*, 1992). Several-fold higher levels of DIF-1 dechlorinase are found at the anterior of slugs (Kay *et al.*, 1993), suggesting that negative feedback pathways may play a role in DIF-1 induction of prestalk genes. Some evidence suggests that cAMP-induced competence for subsequent induction of *ecmB* by DIF-1 is mediated by the cAMP receptor isotype cAR2, which is expressed only in pstA cells (Saxe *et al.*, 1996; Verkerke van Wijk *et al.*, 1998).

Work in a number of laboratories has demonstrated that individual growing cells may have an inherent preference to differentiate into particular cell types. *In vitro* studies, in which cells plated at low density are induced to differentiate, reveal that cells in S or early G2 phase (the *Dictyostelium* cell cycle has no G1 phase) at the time of starvation have a propensity to differentiate into prestalk cells, whereas cells in the rest of the cell cycle express prespore markers (Clay *et al.*, 1995; Gomer and Firtel, 1987; Maeda, 1993; Weijer *et al.*, 1984b). These results occur in isolated cells at low density, suggesting that cell-autonomous mechanisms play an important role in initial cell-type choice and the subsequent regulation of cell-type differentiation (Gomer and Firtel, 1987). Lengthening of S phase using pharmacological agents leads to an increase in the fraction of cells that initially express prestalk markers

(Gomer and Ammann, 1996). Moreover, growing cells synchronized in S or early G2 phase produce organisms with a disproportionate number of prestalk cells when developed as a pure population and tend to differentiate into prestalk cells when mixed with unsynchronized cells (Araki *et al.*, 1994, 1997; McDonald and Durston, 1984; Ohmori and Maeda, 1987; Weijer *et al.*, 1984a). Mid/late-G2-phase cells have a similar tendency to become prespore cells when mixed with unsynchronized cells (Araki *et al.*, 1997; Huang *et al.*, 1997; Wang *et al.*, 1988; Weijer *et al.*, 1984a). A screen for mutations that lead to altered cell-type proportions *in vitro* resulted in the isolation of *RtoA* (Wood *et al.*, 1996). In strains without a functional *RtoA* gene, the fraction of cells that initially express the prestalk-enriched marker CP2 in low-density culture or dissociated aggregates increases from roughly 10 to 15%, although the number of cells that induce a prespore marker gene is not measurably altered. Most strikingly, the correlation between cell cycle position and cell-type choice is lost in *rtoA* null cells; prestalk and prespore cells are randomly derived from cells in any part of the cell cycle. Nonetheless, the cell-type proportioning in *rtoA* null cells is similar to that of wild-type cells, suggesting that alternative mechanisms may regulate initial cell-type choice in this mutant.

The functional link between cell cycle and cell-type differentiation is unclear. Cells examined early in the cell cycle have an increase in gene expression and biochemical activity of the cAMP signaling components needed to initiate aggregation (McDonald, 1986; Wang *et al.*, 1988). It is possible that cell-cycle-coupled regulation of cAMP relay components affects the timing with which cells begin chemotaxing toward cAMP. These temporal differences could allow rapidly aggregating cells to establish morphogen signals that affect cells which enter the developing mound later (Araki *et al.*, 1997; Early *et al.*, 1995; Krefft *et al.*, 1984). Characterization of the promoter elements controlling the cAMP signaling components that are up-regulated early in the cell cycle may elucidate the mechanisms that link the cell cycle to cell-type differentiation.

Growth conditions can also affect cell-fate choices, supporting the model that heterogeneity within populations of vegetative cells may affect initial cell-fate decisions and that the propensity to differentiate into prestalk or prespore cells may be related to cell cycle position at the onset of starvation (Blaschke *et al.*, 1986; Forman and Garrod, 1977; Leach *et al.*, 1973; Tasaka and Takeuchi, 1981).

THE SWITCH BETWEEN AGGREGATION AND CELL-TYPE DIFFERENTIATION

After mound formation, a developmental “switch” occurs. Rising cAMP levels lead to permanent adaptation of aggregation-stage pathways mediated by the high-affinity receptor cAR1 and repression of expression of the components required for aggregation (Abe and Yanagisawa, 1983; Firtel, 1995; Mehdy *et al.*, 1983; Town and Gross, 1978).

This same receptor-saturating dose of cAMP leads to the activation of another set of cAMP-receptor-dependent pathways (Kimmel and Firtel, 1991; Loomis, 1996; Schnitzler *et al.*, 1994; Williams, 1991). In contrast to most but not all aggregation-stage pathways, the ones induced in the mound by cAMP through cAR1 are G-protein independent and lead to the activation of the transcription factors GBF (G-box binding factor) and Dd-STATa (Fig. 2) (Araki *et al.*, 1998; Brown *et al.*, 1997; Kawata *et al.*, 1997; Schnitzler *et al.*, 1995). GBF binds the G box, an essential *cis* regulatory element found in the promoters of postaggregative and cell-type-specific genes (Ceccarelli *et al.*, 1992; Datta and Firtel, 1988; Fosnaugh and Loomis, 1993; Hjorth *et al.*, 1989, 1990; Pears and Williams, 1988; Powell-Coffman *et al.*, 1994). In the absence of GBF, no further cell-type differentiation can occur, as GBF function is required for the induction of all postaggregative and cell-type-specific genes examined (Schnitzler *et al.*, 1994, 1995).

Dd-STATa is rapidly tyrosine phosphorylated and translocated to the nucleus in all mound-stage cells in response to cAMP (Araki *et al.*, 1998). By the slug stage, Dd-STATa nuclear localization is high only in the very anterior of the *pstA* domain; however, some *pstO* cells and ALCs show weaker Dd-STATa nuclear localization. Little or no Dd-STATa is found in prespore cell nuclei. *In vitro*, Dd-STATa protein binds to an activating element in the *ecmA* promoter and to two repressor elements in the *ecmB* promoter (Harwood *et al.*, 1993; Kawata *et al.*, 1996). Mutation of the *ecmA* promoter-activating element leads to loss of both Dd-STATa DNA binding and *ecmA/lacZ* expression. In contrast, mutation of the *ecmB* repressor sites, which also causes loss of DNA binding, results in ectopic expression of *ecmB/lacZ* throughout the entire prestalk domain and in ALCs. Analysis of *Dd-STATa* null strains demonstrates that *ecmB* is expressed throughout the prestalk region, similar to observations upon deletion of the *ecmB* repressor elements. These data suggest that Dd-STATa binding to the *ecmB* promoter is responsible for *ecmB* repression during the slug stage (Mohanty *et al.*, 1999). *ecmA* is expressed in *Dd-STATa* null cells, although some spatial patterning defects are observed. Whereas *ecmA/lacZ* is expressed normally, *ecmO/lacZ* staining is seen throughout the prestalk domain at the onset of slug formation. This may result from the physical inability of prestalk cell types to efficiently sort out from each other, as *Dd-STATa* null cells have cell movement defects during aggregation. Alternatively, the altered expression of *ecmB* and possibly other genes may inhibit the proper specification of prestalk cell types or an inability to recognize signals required for cell-type segregation. Interestingly, *Dd-STATa* null strains fail to produce stalk cells *in vivo*, possibly due to the hypersensitivity of *Dd-STATa* null cells to cAMP-mediated repression of stalk cell differentiation, a phenomenon that occurs in wild-type cells (Berks and Kay, 1988).

Although only Dd-STATa has been analyzed in detail, two other *Dictyostelium* STAT proteins have been identified (J. Williams, personal communication; Mohanty *et al.*,

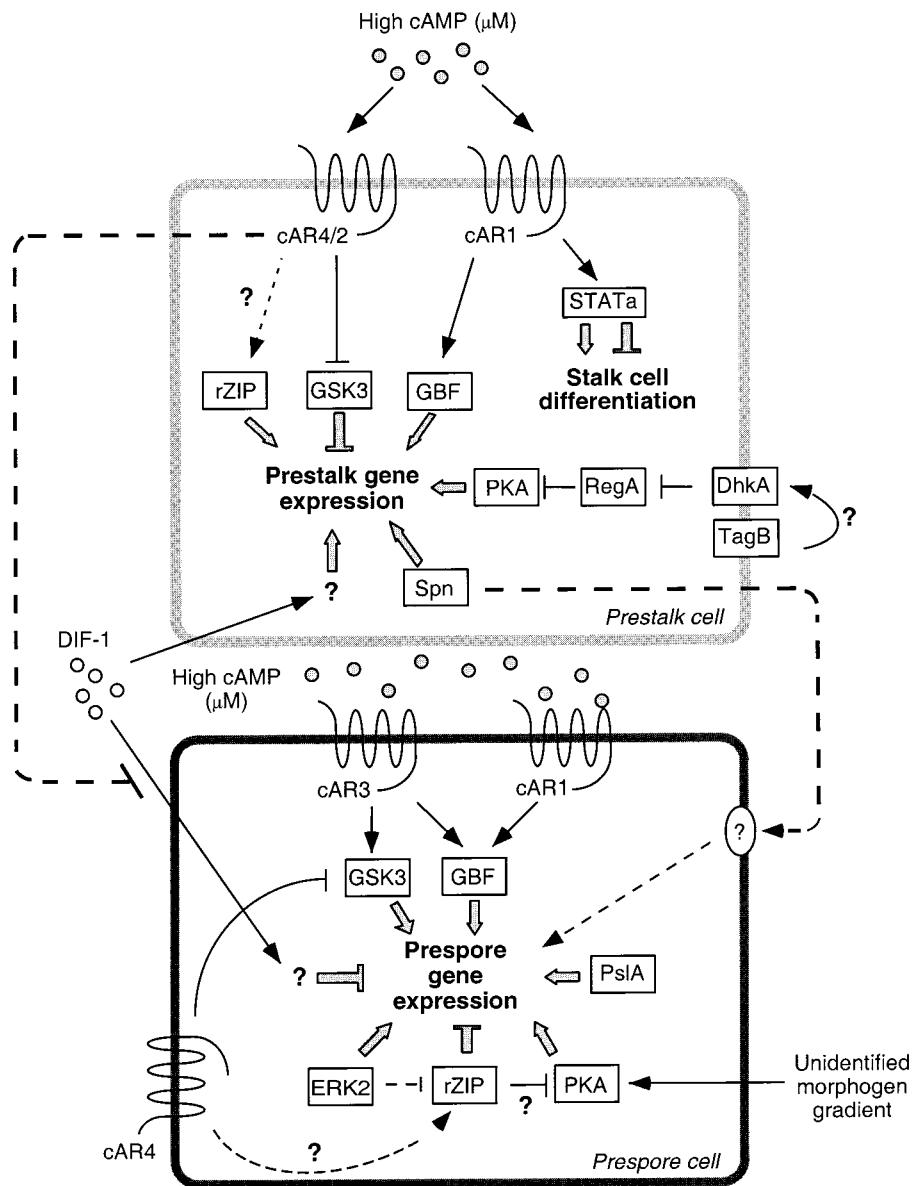


FIG. 2. Proteins and extracellular factors controlling cell-type differentiation. Prestalk and prespore gene expression are regulated by various extracellular factors. cAMP functions through the high-affinity receptors cAR1 and cAR3 and the low-affinity receptors cAR2 and cAR4. The receptor for DIF-1, a small, membrane-permeable molecule, has not yet been identified, but is expected to be intracellular. Experiments with *spn* null and *cAR4* null strains suggest that prestalk cells nonautonomously regulate prespore gene expression. An unidentified morphogen gradient that affects PKA activity is suggested by results obtained using *rzpA* null and wild-type cell chimeras. TagB may be involved in the production of an autocrine peptide signal that stimulates the differentiation of prestalk cells. The receptor for such a peptide is unknown, but may be a transmembrane histidine kinase similar to DhkA. STATa appears to play a dual role in stalk cell formation. Expression of stalk cell markers is derepressed in *STATa* null cells; however, these cells remain unable to complete terminal stalk cell differentiation. See text for details and references.

1999). Because *Dd-STATa* null nuclear extracts contain a DNA-binding activity to the *ecmA* promoter-activating element, it is possible that one of these proteins may be responsible for induction of *ecmA*. Given the lack of STATs

in the genomes of *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, *Dictyostelium* provides the simplest system in which to examine these important signaling molecules.

TABLE 1

Summary of Developmentally Important Genes Discussed in Text

Gene	Homology	Expression pattern	Effect of null on prespore population	Effect of null on prestalk population	Notes
GBF	Zn-finger transcription factor	All cells	No gene expression	No gene expression	No postaggregative or cell-type-specific gene expression
Dd-STATa	STAT	All cells	Decreased prespore domain in slug	Increased pstO, pstB	No stalk cell differentiation <i>in situ</i> , but occurs <i>in vitro</i>
Spn	G α subunit, PP2C	Prestalk, ALC	No gene expression (nonautonomous)	No gene expression (autonomous)	Postaggregative genes expressed (GBF, LagC)
G α 4	G α subunit	ALC	Decreased gene expression	No effect	
Ps1A	None	ND	No gene expression	Decreased ecmA, increased ALC	Nuclear localized
TagB	Serine protease, MDR transporter	Prestalk	No effect	Decreased ecmA	
cAR2	Seven-span receptor	PstA	Increased gene expression	Increased ecmA, decreased ecmB	Probably negatively regulates GskA
cAR3	Seven-span receptor	All cells	Decreased pspA	Increased ecmB	Positively regulates GskA
cAR4	Seven-span receptor	Prestalk	Increased gene expression	Decreased ecmA, ecmB	Probably negatively regulates GskA
GskA	GSK-3	Probably all cells	Decreased pspA	Increased ecmB	Classes of prespore genes are differentially regulated by PKA
PKA-C	PKA catalytic subunit	All cells	No SP60/cotC, but pspA expressed normally	Reduced ecmA	
Erk2	Map kinase	ND	No gene expression	No effect	May also regulate A/P gradient of PKA activity
rZIP	Ring finger, SH3 domain, leucine zipper	All cells	Increased gene expression	Decreased ecmA, ecmB	
Wri	Homeobox	PstA	Decreased prespore domain in slug	Increased pstO domain in slug	Compartment border maintained
MEKK α	MAP kinase kinase (contains an F box and WD40 repeats)	ND	Decreased prespore domain in slug	Increased pstO domain in slug	Compartment border lost
FbxA	WD40/F box-containing protein	ND	Increased prespore domain in slug	Decreased pstO domain in slug	
HP1	Unknown	ND	Decreased prespore gene expression	Increased ecmB	Gene has not been cloned

Note. Effects on prespore and prestalk differentiation refer to null mutants for most genes. Studies on PKA-C have utilized a dominant negative mutant regulatory subunit (PKA-Rm) and studies on ERK2 have utilized a temperature-sensitive mutant because of the inability of these two null strains to aggregate. HP1 is a chemically induced mutation and the nature of the genetic lesion is not known. ND, not determined.

EARLY STEPS IN CELL-TYPE DIFFERENTIATION

New insights into the mechanisms controlling cell-fate decisions have been obtained through genetic and molecular genetic analyses of the requirements for cell-type-specific gene expression. Table 1 presents a summary of the

genes discussed throughout the text, their functions, and phenotypes of their null mutations. Mutational analysis of the early steps of cell-type differentiation presents some evidence that the initial differentiation of prestalk cells is required for proper induction of the prespore cell population. Spalten (Spn), which contains a G-protein α subunit-like domain coupled to a PP2C-like phosphatase domain, is

required for both prestalk and prespore cell-type differentiation (Fig. 2) (Aubry and Firtel, 1998). Cells lacking Spn arrest as tight mounds before breaking up into smaller aggregates. Spn appears to be expressed in prestalk cells and ALCs during multicellular development. Expression of the Spn phosphatase domain alone is sufficient to restore prestalk cell differentiation in *spn* null cells, indicating that this is the effector domain, although development of these strains is not normal. This finding suggests that dephosphorylation of a specific Spn substrate(s) is a limiting step in the differentiation of prestalk cells. Mutational analysis of the G α -like domain indicates that the activity of the PP2C domain is regulated by the G α -like domain, at least in part, by guanine nucleotides. A second-site suppressor that allows *spn* null cells to form fruiting bodies has been identified (L. Aubry and R.A.F., unpublished observations). This gene (ARCK1) encodes a kinase with a domain structure similar to metazoan Raf-1 and contains ankyrin repeats. ARCK1 may compete with Spn for a common substrate or could be a target for Spn phosphatase activity. In chimeric organisms with wild-type or *psIA* null cells, a strain that is unable to differentiate into prespore cells, *spn* null cells form prespore/spore cells but not prestalk/stalk cells, suggesting that the defect in prespore cell specification is nonautonomous (Aubry and Firtel, 1998; Yasukawa *et al.*, 1998). Spn may be required for the initial formation of prestalk cells and/or ALCs, which induce neighboring cells to adopt a prespore fate.

The production of a prespore cell induction factor by prestalk cells has previously been proposed by studies of the heterotrimeric G-protein subunit G α 4 (Hadwiger *et al.*, 1994), which is highly enriched in ALCs during multicellular development (Hadwiger and Firtel, 1992). Initial prestalk cell differentiation is normal in *ga4* null cells, but prespore gene expression is delayed and decreased compared to the parental strain and very few viable spores are produced in *ga4* null cells. Both of these defects are partially rescued in chimeric mixtures of *ga4* null cells with wild-type or G α 4-overexpressing cells, suggesting that G α 4 may be involved in the production of a prespore induction factor by the ALC population. The identity of such a factor is unknown, although putative prespore induction factors have been reported (Kumagai and Okamoto, 1986; Oohata *et al.*, 1997). Studies on cells lacking the prestalk-enriched cAMP receptor cAR4 (described below) suggest that prestalk cells secrete a factor that modulates prespore gene expression, but it is not required for the induction of prespore cell differentiation (Ginsburg and Kimmel, 1997).

Another recently characterized gene, *PsIA*, is required for the differentiation of prespore cells (Yasukawa *et al.*, 1998). *PsIA* has no obvious homology to known proteins, but it localizes to the nucleus, suggesting it is involved in controlling gene expression. Cells lacking *PsIA* make long aggregation streams with tips forming along their length. These tips extend into fingers and differentiate into stalk cells but leave ~50% of the total population behind as undifferentiated cells. No prespore or spore gene expression

is detected either in developing cells or in response to *in vitro* stimulation with cAMP. *psIA* null cells show a clear difference from *spn* null cells: they fail to enter the prespore pathway when codeveloped with wild-type cells, indicating that *PsIA* plays a cell-autonomous role in prespore differentiation. Prestalk patterning is affected in *psIA* null organisms as well. *ecmA*O/*lacZ* expression is confined to a smaller anterior compartment and *ecmO*/*lacZ* and the ALC marker G α 4/*lacZ* are misexpressed in a large fraction of cells throughout the organism, most notably in the pstA zone. *PsIA* may be required in prestalk cells for perception of a negative feedback signal that helps establish the equilibrium required for the proper proportioning of cell types. Alternatively, this could be an indirect effect, owing to the lack of prespore cells, which could be the source of such a signal. The altered patterning may be partially due to morphological abnormalities caused by the aggregation defects.

Development past the tight mound stage requires TagB, a prestalk-specific transmembrane protein containing a serine protease domain and an ATP-driven transporter domain similar to that found in multidrug-resistance genes (Shaulsky *et al.*, 1995). *tagB* null cells have greatly reduced *ecmA* expression, whereas prespore gene expression appears unaffected. A detailed study of spatial patterning showed weak expression of *ecmA*O/*lacZ*, with stained cells properly localized at the top of the mound. No detectable *ecmO*/*lacZ* expression was observed, although this may not be unexpected considering this promoter is expressed more weakly than *ecmA*O/*lacZ*. *tagB* null cells make very little DIF-1. However, it is not clear what the linkage, if any, is between the lack of DIF-1 in *tagB* null cells and the inability of this mutant to induce normal levels of prestalk gene expression (Shaulsky and Loomis, 1996). Although some *ecmA*O/*lacZ* expression is restored to developing *tagB* null cells by DIF-1 treatment, the morphological defects are not rescued. Chimeric development of *tagB* null cells with wild-type cells restores the ability of *tagB* null cells to participate in fruiting body formation. Interestingly, *tagB* null cells are found only in the pstO and ALC populations of such chimeras and seem to be excluded from the anteriormost pstA domain. The phenotypes suggest that the initial commitment of starving cells to the prestalk pathway does not require TagB, but the proper differentiation and possibly the divergence of prestalk subtypes may depend on TagB function. The domain structure of the TagB protein is consistent with its involvement in the export of a peptide signal. There is evidence that this is the case late in development when TagC, a protein highly homologous to TagB, is required in prestalk cells for the production of SDF-2, a peptide that induces terminal differentiation of spore cells (Anjard *et al.*, 1998b). Binding of SDF-2 to DhkA, a two-component histidine kinase receptor found on the surface of prespore cells, leads to the down-regulation of the intracellular, cAMP-specific phosphodiesterase RegA via its response-regulator domain (Wang *et al.*, 1999). As a result, cAMP levels rise, leading to the activation of PKA, which

induces terminal spore differentiation. SDF-2 is thought to simultaneously activate PKA-dependent positive feedback pathways in prestalk cells that cause continued SDF-2 secretion (Anjard *et al.*, 1998a,b). In a similar manner, TagB may be involved in the production of a peptide signal required for the differentiation of prestalk cells in the mound. As maximal induction of prestalk cell differentiation requires PKA, an autocrine signaling system like that involved in SDF-2 secretion could control prestalk-specific pathways by modulating the activity of PKA at the mound stage (Fig. 2). In the absence of TagB, high RegA activity may maintain low levels of intracellular cAMP, which would prevent the PKA-dependent expression of prestalk-specific genes.

Although expression of *ecmA*, the marker most often used to identify prestalk cells, is dependent on DIF-1 *in vitro*, examination of *TagB* and *cAR2*, a cAMP-receptor isotype specifically found in prestalk cells, indicates that expression of these genes may not require DIF-1. Expression of a *TagB* promoter/*lacZ* fusion and sorting of prestalk cells to the apex of mounds are observed in *tagB* null cells, even though these cells produce very low levels of DIF-1 compared to wild-type cells (Shaulsky and Loomis, 1996). *cAR2*, which is expressed only in the anterior *pstA* domain of developing wild-type organisms, is repressed rather than induced by DIF-1 *in vitro* (Saxe *et al.*, 1996). These results suggest that, although a high level of DIF-1 mediates progression along the prestalk pathway, some early prestalk differentiation events may be DIF-1 independent or require only low levels of DIF-1. The genetic lesion(s) in the DIF-1-deficient strain HM44 has not been identified (Kopachik *et al.*, 1983). Targeted mutagenesis of genes required for DIF-1 biosynthesis may help resolve this question (Kay, 1998).

REGULATION OF CELL-TYPE DIFFERENTIATION

Insights into the mechanisms controlling cell-type divergence have been obtained from mutants that retain the ability to generate prestalk and prespore cells but have altered proportioning due to cell-autonomous defects in cell-type differentiation pathways. As the role of extracellular cAMP in the coordination of cell-type-specific gene expression is well-established, it is not surprising that mutations in cAMP receptor genes lead to differentiation and patterning defects (Ginsburg *et al.*, 1995; Rogers *et al.*, 1997). *cAR1*, the primary chemoattractant receptor regulating aggregation, is expressed throughout development (Klein *et al.*, 1988; Louis *et al.*, 1993; Saxe *et al.*, 1991; Sun and Devreotes, 1991). Cells that do not express the cAMP receptor isotype *cAR2* arrest at mound stage without forming a tip (Saxe *et al.*, 1993). *cAR2* is expressed in *pstA* cells early in mound formation (Saxe *et al.*, 1996). The prestalk markers *ecmA* and *ecmB* are expressed in *cAR2* null strains, although *ecmB* expression is somewhat reduced

and expression of the prespore marker *pspA* is 10-fold higher than in wild-type cells. As the prestalk population constitutes only ~20% of the organism, this increase cannot solely be due to conversion of prestalk cells into prespore cells. Comparison of receptor isotype function in cell culture shows that *cAR2* has a much stronger capacity than either *cAR1* or *cAR3* to confer cAMP-mediated competence for prestalk gene induction by DIF-1 (Verkerke van Wijk *et al.*, 1998). Combined with the observation that *cAR2* induction may be DIF-1 independent, this result suggests that *cAR2* expression may be an early step in the establishment of the prestalk cell population, which may be dependent on DIF-1 for cell-type differentiation. However, *cAR2* is not essential for the ability of cells to express prestalk markers (Saxe *et al.*, 1993).

Gene expression defects similar to those found in *cAR2* null cells are observed in cells lacking *cAR4*, whose expression is highly enriched in prestalk cells (Louis *et al.*, 1994). Prespore markers are markedly overexpressed in *cAR4* null cells, whereas the expression of *ecmA* and *ecmB* is severely reduced. Unlike *cAR2* null cells, cells deficient in *cAR4* complete development, but slug and fruiting body morphology are very abnormal and prespore cells are found in the anterior, normally prestalk region, of slugs. The defects are not rescued by exogenous cAMP and/or DIF-1 in cell culture, suggesting that, although *cAR4* is highly enriched in prestalk cells, *cAR4* plays a cell-autonomous role in gene expression in both cell types (Ginsburg and Kimmel, 1997). *cAR4* appears to positively regulate prespore gene expression in a cell-nonautonomous manner. Medium conditioned by wild-type cells contains a secreted factor missing from conditioned medium from *cAR4* null cells that antagonizes the ability of DIF-1 to repress prespore gene expression in suspension. This observation may help explain why, although DIF-1 represses *SP60/cotC* expression in cell culture experiments, prespore-specific gene expression is not inhibited in the posterior of migrating slugs even though the DIF-1 concentration is at least double that found in the prestalk domain (Brookman *et al.*, 1987; Kay *et al.*, 1993).

Studies on *gskA*, the *Dictyostelium* homologue of GSK-3, reveal that, as in higher eukaryotes, this Ser/Thr kinase plays an important role in cell-fate decisions (Bourious *et al.*, 1990; Harwood *et al.*, 1995; He *et al.*, 1995; Siegfried *et al.*, 1992). cAMP signaling through multiple receptors appears to be integrated to precisely control the level of GSKA activity in each cell (Plyte *et al.*, 1999) (Fig. 2). In *gskA* null cells, expression of *ecmB* is significantly increased and *ecmB/lacZ* staining is seen throughout developing mounds, whereas the number of cells expressing the prespore-specific gene *pspA* is reduced. As a result, terminally developed *gskA* null organisms contain large basal structures and most cells differentiate into basal disk/stalk cells. These studies and *in vitro* cell culture experiments indicate that GSKA is required for repression of stalk cell formation and normal induction of prespore gene expression, both of which occur in response to cAMP (Berks and Kay, 1988,

1990). Treatment of wild-type cells with LiCl, which inhibits GSKA, causes defects similar to those seen in *gskA* null cells (Maeda, 1970; Van Lookeren Campagne *et al.*, 1988). LiCl treatment also blocks the conversion of prestalk cells into prespore cells in dissected slugs, further supporting the hypothesis that GSKA is required for prespore differentiation and negatively regulates prestalk pathways (Sakai, 1973).

Comparison of *gskA* null phenotypes to various cAMP-receptor mutant strains reveals similarities to *cAR3* null cells (Plyte *et al.*, 1999). Like *gskA* null cells, *cAR3* null cells have a dramatic increase in *ecmB/lacZ* staining and a reduction in *pspA* expression at mound stage. As described above, wild-type prestalk cells differentiate into stalk cells when treated with DIF-1 *in vitro*, but this process is inhibited by the presence of cAMP (Berks and Kay, 1988). This does not occur in either *gskA* or *cAR3* null cells. Overexpression of *cAR3* in *gskA* null cells does not restore cAMP repression of stalk differentiation, suggesting GSKA lies downstream of *cAR3*. Moreover, *cAR3* is required for cAMP-stimulated increase in GSKA kinase activity, consistent with this model. The cell-type differentiation and morphological defects in *cAR3* null cells are much less severe than in *gskA* null cells, possibly due to the basal activity of GSKA or the ability of another cAMP receptor, presumably *cAR1*, to partially compensate for the lack of *cAR3* function (Johnson *et al.*, 1993; Plyte *et al.*, 1999).

Experiments with other cAMP-receptor mutants hint at the complexity of signaling through GSKA. *gskA* null cells exhibit phenotypic effects opposite those of *cAR2* and *cAR4* null cells on cell-type-specific gene expression (Harwood *et al.*, 1995; Louis *et al.*, 1994; Saxe *et al.*, 1993). Treatment of *cAR4* null cells with increasing concentrations of LiCl gradually restores prestalk and prespore gene expression to wild-type levels, indicating that *cAR4* may negatively regulate GSKA (Ginsburg and Kimmel, 1997). *In vitro* cAMP repression of stalk cell differentiation is more efficient in *cAR2* null than in wild-type cells, suggesting that *cAR2* may negatively regulate GSKA (Plyte *et al.*, 1999).

Other protein kinases play prominent roles in *Dictyostelium* development. PKA and the MAP kinase ERK2 are essential elements of the cAMP relay circuit during aggregation and are required for cell-type-specific gene expression (Gaskins *et al.*, 1996; Harwood *et al.*, 1992; Mann *et al.*, 1997; Mann and Firtel, 1991; Schulkes and Schaap, 1995; Segall *et al.*, 1995; Simon *et al.*, 1992). PKA plays an essential role in the differentiation of prespore and prestalk cells (Fosnaugh and Loomis, 1991; Mann and Firtel, 1993). Expression of a dominant negative PKA-regulatory subunit, which is unable to bind cAMP, in either cell type leads to a block in differentiation (Hopper *et al.*, 1993, 1995; Zhukovskaya *et al.*, 1996). Although cells lacking the PKA-catalytic subunit (*pka-cat* null cells) are unable to aggregate, cell-type-specific gene expression in response to cAMP can be examined in cell culture (Harwood *et al.*, 1992; Mann *et al.*, 1997; Mann and Firtel, 1991). Expression of some prespore markers, such as *pspA*, is not dependent on

PKA (Hopper *et al.*, 1993). In addition, overproduction of GBF in *pka-cat* null cells at least partially restores the ability to express *ecmA* at near normal levels in response to cAMP, suggesting that alternative PKA-independent parallel pathways exist in both cell types (Mann *et al.*, 1997).

Throughout development, PKA activity is modulated by proteins that affect cytoplasmic cAMP concentrations (Devreotes, 1994; Firtel, 1995; Loomis, 1998). Signals that induce cAMP production by activating adenylyl cyclase enzymes are antagonized by pathways that stimulate the cAMP-specific cytoplasmic phosphodiesterase RegA (Brown and Firtel, 1998; Chang *et al.*, 1998; Shaulsky *et al.*, 1996, 1998; Soderbom and Loomis, 1998; Thomason *et al.*, 1998). Cytoplasmic cAMP concentrations may also be controlled through ERK2, which in genetic analyses appears to inhibit RegA function (B. Wang and A. Kuspa, personal communication).

The function of PKA in cell-type differentiation appears to depend in part on rZIP, a ubiquitously expressed adaptor protein containing a RING finger, SH3 domain, leucine zipper, and glutamine-rich repeat (Balint-Kurti *et al.*, 1997). *rzpA* null cells have a slight reduction in *ecmA* and *ecmB* expression and a three- to fivefold increase in prespore gene expression. In addition, no scattered *ecmA/lacZ* or *ecmB/lacZ* staining is observed in the prespore zone, suggesting a defect in ALC differentiation. Overexpression of rZIP causes the opposite effect: expression of prestalk genes is up-regulated and prespore gene expression is strongly repressed. Mosaic experiments using *rzpA* null and wild-type cells have yielded intriguing results (Balint-Kurti *et al.*, 1998). The prespore marker *SP70/cotB*, which is responsive to PKA, is expressed throughout the posterior in both strains. However, when a small fraction (10%) of *rzpA* null cells is codeveloped in chimeras with wild-type cells, only the rZIP null cells in the anterior part of the prespore zone express *SP70/cotB*, even though mutant cells are evenly distributed throughout this domain. Importantly, *pspA/lacZ*, whose induction is PKA independent, is expressed in all *rzpA* null prespore cells of the chimera. Expression of *SP70/cotB* in the whole prespore compartment is restored by treatment with the membrane-permeable PKA activator 8-Br-cAMP, implying that *rzpA* null cells require a higher level of PKA activity to express PKA-dependent prespore markers and that PKA activity is higher in the anteriormost cells of the prespore domain of these chimeras. Heterogeneity within the prespore population has been previously reported. Disaggregated prespore cells return to the region of the prespore domain from which they originated when allowed to form new slugs (Buhl *et al.*, 1993). Furthermore, expression of *lacZ* from an *SP60/cotC* promoter from which the two 5'-distal of three G boxes (GBF binding sites) have been deleted results in a pattern of expression similar to that seen in the *rzpA* null cells in chimeras with wild-type cells (Haberstroh and Firtel, 1990; Haberstroh *et al.*, 1991). Expression of *lacZ* from this altered promoter is seen in the entire prespore domain of *rzpA* null cells developed alone. The results suggest that, in addition to affecting the thresh-

old of PKA activity required to induce prespore gene expression, rZIP may regulate the level of a graded anterior-posterior signal that controls prespore gene expression by modulating the activity of PKA (Fig. 2). The relationship of this putative gradient to the cAR4-dependent secreted factor is unknown. It is interesting to note that, although cAR4 and *rzpA* null strains have a similar increase in prespore gene expression, it appears that in wild-type cells, cAR4 and rZIP function antagonistically in the nonautonomous regulation of prespore differentiation.

CELL-TYPE PROPORTIONING

One model to explain the reportioning of dissected slugs is mutual inhibition in which both prestalk and prespore cells secrete diffusible factors that prevent cells of the other fate from switching cell types (Loomis, 1993; Soderbom and Loomis, 1998). Removal of either population would lead to derepression and redifferentiation until enough cells have switched fates to reach equilibrium. However, the mechanisms governing cell-type proportioning *in vivo* are likely to be more complex. After removal of the prestalk domain, ALCs rapidly migrate to the anterior, differentiate into prestalk cells, and are replenished by dedifferentiation of cells from the prespore population and their subsequent conversion to ALCs (Abe *et al.*, 1994; Sternfeld, 1992; Sternfeld and David, 1982). This process has been termed "transdifferentiation." In addition, no *ecmA*O/*lacZ* or *ecmB*/*lacZ* staining is apparent in the posterior of *rzpA* null cells, suggesting a defect in ALC formation (Balint-Kurti *et al.*, 1997). When prestalk and prespore domains of *rzpA* null slugs are separated, the posterior section is unable to form a fruiting body, suggesting that proper ALC differentiation is required for efficient conversion of prespore into prestalk cells. Conversion of prespore cells to ALCs, ALCs to pstO cells, and pstO to pstA cells is observed during normal slug migration (Abe *et al.*, 1994; Detterbeck *et al.*, 1994; Sakai, 1973; Shaulsky and Loomis, 1993). During slug migration, some pstAB cells prematurely enter the stalk differentiation pathway and shed from the back of the slug (Sternfeld, 1992). This population is regenerated by the conversion of some pstA cells to pstAB cells and their entry into the cone at the anterior of the slug. Therefore, proportioning of cell types is likely to be a result of equilibria between several cell types (pstA \leftrightarrow pstO \leftrightarrow ALC \leftrightarrow prespore) (Blaschke *et al.*, 1986; MacWilliams *et al.*, 1985).

Mutational analysis suggests that the control of cell-type proportions is complex and involves multiple regulatory pathways, including the function of two homeobox-containing transcription factors (Han and Firtel, 1998). Disruption of one of these genes, *Wari*i (*Wri*), causes the size of the pstO domain to more than double, with an accompanying decrease in the prespore compartment. The pstA domain is unaffected in *wri* null slugs. Interestingly, *Wri* appears to be expressed primarily in pstA cells and the

wri null phenotype can be rescued by *Wri* expression from the pstA-specific *ecmA* promoter but not the *ecmO* or prespore promoters. In mosaic experiments, *wri* null cells induce an expansion of *ecmO*/*lacZ* expression in a few codeveloped wild-type cells, further supporting the notion that the *wri* null defect is cell nonautonomous. Although it has not yet been determined if *wri* null cells initially form too many pstO cells, the fact that altered ratios are seen after slug migration suggests that *wri* null cells have a defect in the homeostasis mechanisms that maintain cell-type proportions. Because it is difficult to accurately measure the number of ALCs, it is unclear whether the prespore \leftrightarrow ALC, ALC \leftrightarrow pstO, or both equilibria are disturbed.

In cells containing a mutation in the MAP kinase kinase kinase, MEKK α , a similar alteration is observed (Chung *et al.*, 1998). The pstO domain is expanded and the prespore domain is reduced; however, unlike *wri* null slugs, the sharp border between the compartments is lost. PstO cells and prespore cells are intermingled, although no cells appear to express both markers. In mosaic experiments with wild-type cells, *mekk* α null cells appear to initially express prespore genes. As development proceeds, this expression is lost and presumably these cells differentiate into prestalk cells, suggesting MEKK α function is involved in maintenance of prespore identity. In accord with this finding, cells overexpressing MEKK α form the majority of the prespore population when codeveloped with wild-type cells. The results indicate that MEKK α may influence each cell's sensitivity to extracellular morphogen factors. A higher level of MEKK α activity seems to shift the balance of cell-type proportions toward the prespore fate at the expense of the pstO/ALCs.

MEKK α has a C-terminal F box and WD40 repeats that target the protein to the cell cortex. In addition, these motifs target MEKK α for ubiquitin-mediated degradation via the conjugating enzyme UbcB. MEKK α is stabilized from degradation by the deubiquitinating enzyme UbpB. Degradation of a constitutively expressed GFP-F box/WD40 fusion protein is spatially and temporally regulated in whole organisms. This GFP-fusion protein is found preferentially in prestalk cells and is degraded in prespore cells. Very little GFP-fusion protein is seen in any part of *ubpB* null slugs and ectopic expression of UbpB in prespore cells results in GFP-F box/WD40 protein stability throughout the organism. Another F-box/WD40-repeat-containing protein, FbxA, which contains a novel N-terminal domain, has null and overexpression phenotypes that are the opposite of those of MEKK α mutant strains (M. K. Nelson, A. Clark, T. Abe, A. Nomura, N. Yadava, C. J. Funair, K. A. Jermyn, R. A. Firtel, and J. G. Williams, in preparation). One possibility is that FbxA lies downstream of MEKK α and MEKK α regulates FbxA function.

Another mutant, HP1, displays a conversion of prespore cells to prestalk pathways (Bichler and Weijer, 1994). The genetic lesion in this strain has not been identified. Careful examination of developing HP1 cells shows that many cells initially express prespore genes but subsequently change

cell types and begin expressing *ecmB/lacZ*. Initial patterning appears normal in this strain and cells that switch fates remain in the posterior zone, suggesting that they may function as ALCs. A small fraction of wild-type cells codeveloped in a primarily HP1 slug properly differentiate into prespore cells. This result indicates that HP1, in contrast to *wri* null cells, has a cell-autonomous defect in sensitivity to extracellular signals that stabilize prespore identity. Because it was isolated in a screen for strains that differentiate in the presence of 5 mM caffeine, which inhibits adenylyl cyclase activation, HP1 is thought to contain a defect in cAMP signaling. As cAMP is required for maintenance of prespore gene expression and repression of *ecmB*, the HP1 phenotype could be due to a reduced sensitivity to cAMP. This possibility has not yet been directly tested. The phenotype of HP1 suggests a genetic relationship to *gskA* and *cAR3* null cells, which have similar characteristics.

FUTURE DIRECTIONS

Although much has been learned about the processes governing cell-type induction and differentiation in *Dictyostelium*, many questions remain. An *in vitro* analysis of early aggregation-stage gene promoters may shed light on the mechanisms by which the cell cycle influences initial cell-type choice. Further mutagenesis, including the isolation of a strain blocked in DIF-1 biosynthesis, will elucidate the early steps in prestalk cell differentiation. Intercellular induction of prespore cells by the prestalk population will be clarified by the further purification and characterization of extracellular factors produced by prestalk cells. Further mutational analysis and identification of secreted factors will be necessary to determine the mechanisms that control the equilibria between cell types.

Highly conserved molecules have been found to play an integral role in *Dictyostelium* development. The rapid production of genomic information from *Dictyostelium* and other organisms is likely to reveal many more similarities between the cell-type induction and maintenance mechanisms of *Dictyostelium* and higher eukaryotes.

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REFERENCES

- Abe, K., and Yanagisawa, K. (1983). A new class of rapid developing mutants in *Dictyostelium discoideum*: Implications for cyclic AMP metabolism and cell differentiation. *Dev. Biol.* **95**, 200–210.
- Abe, T., Early, A., Siegert, F., Weijer, C., and Williams, J. (1994). Patterns of cell movement within the *Dictyostelium* slug revealed by cell type-specific, surface labeling of living cells. *Cell* **77**, 687–699.
- Anjard, C., Chang, W. T., Gross, J., and Nellen, W. (1998a). Production and activity of spore differentiation factors (SDFs) in *Dictyostelium*. *Development* **125**, 4067–4075.
- Anjard, C., Zeng, C., Loomis, W. F., and Nellen, W. (1998b). Signal transduction pathways leading to spore differentiation in *Dictyostelium discoideum*. *Dev. Biol.* **193**, 146–155.
- Araki, T., Abe, T., Williams, J. G., and Maeda, Y. (1997). Symmetry breaking in *Dictyostelium* morphogenesis: Evidence that a combination of cell cycle stage and positional information dictates cell fate. *Dev. Biol.* **192**, 645–648.
- Araki, T., Gamper, M., Early, A., Fukuzawa, M., Abe, T., Kawata, T., Kim, E., Firtel, R. A., and Williams, J. G. (1998). Developmentally and spatially regulated activation of a *Dictyostelium* STAT protein by a serpentine receptor. *EMBO J.* **17**, 4018–4028.
- Araki, T., Nakao, H., Takeuchi, I., and Maeda, Y. (1994). Cell-cycle-dependent sorting in the development of *Dictyostelium* cells. *Dev. Biol.* **162**, 221–228.
- Aubry, L., and Firtel, R. A. (1998). Spalten, a protein containing G α -protein-like and PP2C domains, is essential for cell-type differentiation in *Dictyostelium*. *Genes Dev.* **12**, 1525–1538.
- Aubry, L., and Firtel, R. A. (1999). Integration of signaling networks that regulate *Dictyostelium* differentiation. *Annu. Rev. Cell Dev. Biol.* **15**, 469–517.
- Balint-Kurti, P., Ginsburg, G., Liu, J., and Kimmel, A. R. (1998). Non-autonomous regulation of a graded, PKA-mediated transcriptional activation signal for cell patterning. *Development* **125**, 3947–3954.
- Balint-Kurti, P., Ginsburg, G., Rivero-Lezcano, O., and Kimmel, A. R. (1997). rZIP, a RING-leucine zipper protein that regulates cell fate determination during *Dictyostelium* development. *Development* **124**, 1203–1213.
- Berks, M., and Kay, R. R. (1988). Cyclic AMP is an inhibitor of stalk cell differentiation in *Dictyostelium discoideum*. *Dev. Biol.* **126**, 108–114.
- Berks, M., and Kay, R. R. (1990). Combinatorial control of cell differentiation by cAMP and DIF-1 during development of *Dictyostelium discoideum*. *Development* **110**, 977–984.
- Berks, M., Traynor, D., Carrin, I., Insall, R. H., and Kay, R. R. (1991). Diffusible signal molecules controlling cell differentiation and patterning in *Dictyostelium*. *Development Suppl.* **1**, 131–139.
- Bichler, G., and Weijer, C. J. (1994). A *Dictyostelium* anterior-like cell mutant reveals sequential steps in the prespore prestalk differentiation pathway. *Development* **120**, 2857–2868.
- Blaschke, A., Weijer, C., and MacWilliams, H. (1986). *Dictyostelium discoideum*: Cell-type proportioning, cell-differentiation preference, cell fate, and the behavior of anterior-like cells in Hs1/Hs2 and G/G- mixtures. *Differentiation* **32**, 1–9.
- Bourious, M., Morre, P., Ruel, L., Grau, Y., Heitzler, P., and Simpson, P. (1990). An early embryonic product of the gene shaggy encodes a serine/threonine protein kinase related to the CDC28/*cdc2*+ subfamily. *EMBO J.* **9**, 2877–2884.
- Bowerman, B. (1998). Maternal control of pattern formation in early *Caenorhabditis elegans* embryos. *Curr. Top. Dev. Biol.* **39**, 73–117.
- Brenner, M. (1977). Cyclic AMP gradient in migrating pseudoplasmodia of the cellular slime mold *Dictyostelium discoideum*. *J. Biol. Chem.* **252**, 4073–4077.

- Brookman, J. J., Jermyn, K. A., and Kay, R. R. (1987). Nature and distribution of the morphogen DIF in the Dictyostelium slug. *Development* **100**, 119–124.
- Brown, J. M., Briscoe, C., and Firtel, R. A. (1997). Control of transcriptional regulation by signal transduction pathways in Dictyostelium during multicellular development. In "Dictyostelium: A Model System for Cell and Developmental Biology" (Y. Maeda, K. Inouye, and I. Takeuchi, Eds.), pp. 245–265. Universal Academy Press, Tokyo.
- Brown, J. M., and Firtel, R. A. (1998). Phosphorelay signalling: New tricks for an ancient pathway. *Curr. Biol.* **8**, R662–R665.
- Buhl, B., Fischer, K., and MacWilliams, H. K. (1993). Cell sorting within the prespore zone of Dictyostelium discoideum. *Dev. Biol.* **156**, 481–489.
- Ceccarelli, A., Mahbubani, H., and Williams, J. G. (1991). Positively and negatively acting signals regulating stalk cell and anterior-like cell differentiation in Dictyostelium. *Cell* **65**, 983–989.
- Ceccarelli, A., Mahbubani, H. J., Insall, R., Schnitzler, G., Firtel, R. A., and Williams, J. G. (1992). A G-rich sequence element common to Dictyostelium genes which differ radically in their patterns of expression. *Dev. Biol.* **152**, 188–193.
- Chang, W. T., Thomason, P. A., Gross, J. D., and Newell, P. C. (1998). Evidence that the RdeA protein is a component of a multistep phosphorelay modulating rate of development in Dictyostelium. *EMBO J.* **17**, 2809–2816.
- Chung, C. Y., Reddy, T. B. K., Zhou, K., and Firtel, R. A. (1998). A novel, putative MEK kinase controls developmental timing and spatial patterning in Dictyostelium and is regulated by ubiquitin-mediated protein degradation. *Genes Dev.* **12**, 3564–3578.
- Clarke, M., Kayman, S. C., and Riley, K. (1987). Density-dependent induction of discoidin-I synthesis in exponentially growing cells of Dictyostelium discoideum. *Differentiation* **34**, 79–87.
- Clay, J. L., Ammann, R. R., and Gomer, R. H. (1995). Initial cell-type choice in a simple eukaryote: Cell-autonomous or morphogen-gradient dependent? *Dev. Biol.* **172**, 665–674.
- Datta, S., and Firtel, R. A. (1988). An 80-bp cis-acting regulatory region controls cAMP and developmental regulation of a prestalk gene in Dictyostelium. *Genes Dev.* **2**, 294–304.
- Davidson, E. H., Cameron, R. A., and Ransick, A. (1998). Specification of cell fate in the sea urchin embryo: Summary and some proposed mechanisms. *Development* **125**, 3269–3290.
- Detterbeck, S., Morandini, P., Wetterauer, B., Bachmair, A., Fischer, K., and MacWilliams, H. K. (1994). The 'prespore-like cells' of Dictyostelium have ceased to express a prespore gene: Analysis using short-lived beta-galactosidases as reporters. *Development* **120**, 2847–2855.
- Devine, K. M., and Loomis, W. F. (1985). Molecular characterization of anterior-like cells in Dictyostelium discoideum. *Dev. Biol.* **107**, 364–372.
- Devreotes, P. N. (1994). G protein-linked signaling pathways control the developmental program of Dictyostelium. *Neuron* **12**, 235–241.
- Dierick, H., and Bejsovec, A. (1999). Cellular mechanisms of wingless/Wnt signal transduction. *Curr. Top. Dev. Biol.* **43**, 153–190.
- Dinauer, M. C., Steck, T. L., and Devreotes, P. N. (1980). Cyclic 3',5'-AMP relay in Dictyostelium discoideum. V. Adaptation of the cAMP signaling response during cAMP stimulation. *J. Cell Biol.* **86**, 554–561.
- Dingermann, T., Reindl, N., Werner, H., Hildebrandt, M., Nellen, W., Harwood, A., Williams, J., and Nerke, K. (1989). Optimization and in situ detection of Escherichia coli beta-galactosidase gene expression in Dictyostelium discoideum. *Gene* **85**, 353–362.
- Dormann, D., Siebert, F., and Weijer, C. J. (1996). Analysis of cell movement during the culmination phase of Dictyostelium development. *Development* **122**, 761–769.
- Dynes, J. L., Clark, A. M., Shaulsky, G., Kuspa, A., Loomis, W. F., and Firtel, R. A. (1994). LagC is required for cell-cell interactions that are essential for cell-type differentiation in Dictyostelium. *Genes Dev.* **8**, 948–958.
- Early, A., Abe, T., and Williams, J. (1995). Evidence for positional differentiation of prestalk cells and for a morphogenetic gradient in Dictyostelium. *Cell* **83**, 91–99.
- Early, A. E., Gaskell, M. J., Traynor, D., and Williams, J. G. (1993). Two distinct populations of prestalk cells within the tip of the migratory Dictyostelium slug with differing fates at culmination. *Development* **118**, 353–362.
- Esch, R. K., and Firtel, R. A. (1991). cAMP and cell sorting control the spatial expression of a developmentally essential cell-type-specific ras gene in Dictyostelium. *Genes Dev.* **5**, 9–21.
- Firtel, R. A. (1995). Integration of signaling information in controlling cell-fate decisions in Dictyostelium. *Genes Dev.* **9**, 1427–1444.
- Firtel, R. A. (1996). Interacting signaling pathways controlling multicellular development in Dictyostelium. *Curr. Opin. Genet. Dev.* **6**, 545–554.
- Fisher, P. R., Dohrmann, U., and Williams, K. L. (1984). Signal processing in Dictyostelium discoideum slugs. In "Modern Cell Biology" (B. H. Satir, Ed.), pp. 197–248. A. R. Liss, New York.
- Forman, D., and Garrod, D. R. (1977). Pattern formation in Dictyostelium discoideum. I. Development of prespore cells and its relationship to the pattern of the fruiting body. *J. Embryol. Exp. Morphol.* **40**, 215–228.
- Fosnaugh, K. L., and Loomis, W. F. (1991). Coordinate regulation of the spore coat genes in Dictyostelium discoideum. *Dev. Genet.* **12**, 123–132.
- Fosnaugh, K. L., and Loomis, W. F. (1993). Enhancer regions responsible for temporal and cell-type-specific expression of a spore coat gene in Dictyostelium. *Dev. Biol.* **157**, 38–48.
- Franke, J., and Kessin, R. H. (1992). The cyclic nucleotide phosphodiesterases of Dictyostelium discoideum: Molecular genetics and biochemistry. *Cell. Signalling* **4**, 471–478.
- Gaskell, M. J., Jermyn, K. A., Watts, D. J., Treffry, T., and Williams, J. G. (1992). Immunolocalization and separation of multiple prestalk cell types in Dictyostelium. *Differentiation* **51**, 171–176.
- Gaskins, C., Clark, A. M., Aubry, L., Segall, J. E., and Firtel, R. A. (1996). The Dictyostelium MAP kinase ERK2 regulates multiple, independent developmental pathways. *Genes Dev.* **10**, 118–128.
- Gaskins, C., Maeda, M., and Firtel, R. A. (1994). Identification and functional analysis of a developmentally regulated extracellular signal-regulated kinase gene in Dictyostelium discoideum. *Mol. Cell. Biol.* **14**, 6996–7012.
- Ginger, R. S., Drury, L., Baader, C., Zhukovskaya, N. V., and Williams, J. G. (1998). A novel Dictyostelium cell surface protein important for both cell adhesion and cell sorting. *Development* **125**, 3343–3352.
- Ginsburg, G. T., Gollop, R., Yu, Y. M., Louis, J. M., Saxe, C. L., and Kimmel, A. R. (1995). The regulation of Dictyostelium development by transmembrane signalling. *J. Eukaryotic Microbiol.* **42**, 200–205.
- Ginsburg, G. T., and Kimmel, A. R. (1997). Autonomous and nonautonomous regulation of axis formation by antagonistic

- signaling via 7-span cAMP receptors and GSK3 in Dictyostelium. *Genes Dev.* **11**, 2112–2123.
- Gomer, R. H. (1998). Antisense: A key tool for cell and developmental studies in Dictyostelium. *Genet. Eng.* **20**, 135–141.
- Gomer, R. H. (1999). Gene identification by shotgun antisense. *Methods Companion Methods Enzymol.* **18**, 311–315
- Gomer, R. H., and Ammann, R. R. (1996). A cell-cycle phase-associated cell-type choice mechanism monitors the cell cycle rather than using an independent timer. *Dev. Biol.* **174**, 82–91.
- Gomer, R. H., Datta, S., and Firtel, R. A. (1986). Cellular and subcellular distribution of a cAMP-regulated prestalk protein and prespore protein in Dictyostelium discoideum: A study on the ontogeny of prestalk and prespore cells. *J. Cell Biol.* **103**, 1999–2015.
- Gomer, R. H., and Firtel, R. A. (1987). Cell-autonomous determination of cell-type choice in Dictyostelium development by cell-cycle phase. *Science* **237**, 758–762.
- Gomer, R. H., Yuen, I. S., and Firtel, R. A. (1991). A secreted 80x10(3)Mr protein mediates sensing of cell density and the onset of development in Dictyostelium. *Development* **112**, 269–278.
- Haberstroh, L., and Firtel, R. A. (1990). A spatial gradient of expression of a cAMP-regulated prespore cell type-specific gene in Dictyostelium. *Genes Dev.* **4**, 596–612.
- Haberstroh, L., Galindo, J., and Firtel, R. A. (1991). Developmental and spatial regulation of a Dictyostelium prespore gene: cis-acting elements and a cAMP-induced, developmentally regulated DNA binding activity. *Development* **113**, 947–958.
- Hadwiger, J. A., and Firtel, R. A. (1992). Analysis of $G\alpha 4$, a G-protein subunit required for multicellular development in Dictyostelium. *Genes Dev.* **6**, 38–49.
- Hadwiger, J. A., Lee, S., and Firtel, R. A. (1994). The $G\alpha$ subunit $G\alpha 4$ couples to pterin receptors and identifies a signaling pathway that is essential for multicellular development in Dictyostelium. *Proc. Natl. Acad. Sci. USA* **91**, 10566–10570.
- Hadwiger, J. A., Natarajan, K., and Firtel, R. A. (1996). Mutations in the Dictyostelium heterotrimeric G protein α subunit $G\alpha 5$ alter the kinetics of tip morphogenesis. *Development* **122**, 1215–1224.
- Han, Z., and Firtel, R. A. (1998). The homeobox-containing gene *Wariai* regulates anterior–posterior patterning and cell-type homeostasis in Dictyostelium. *Development* **125**, 313–325.
- Harwood, A. J., Early, A., and Williams, J. G. (1993). A repressor controls the timing and spatial localisation of stalk cell-specific gene expression in Dictyostelium. *Development* **118**, 1041–1048.
- Harwood, A. J., Hopper, N. A., Simon, M. N., Bouzid, S., Veron, M., and Williams, J. G. (1992). Multiple roles for cAMP-dependent protein kinase during Dictyostelium development. *Dev. Biol.* **149**, 90–99.
- Harwood, A. J., Plyte, S. E., Woodgett, J., Strutt, H., and Kay, R. R. (1995). Glycogen synthase kinase 3 regulates cell fate in Dictyostelium. *Cell* **80**, 139–148.
- He, X., Saint-Jeannet, J. P., Woodgett, J. R., Varmus, H. E., and Dawid, I. B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* **374**, 617–622. [Published erratum appears in *Nature* **375**, 253]
- Hjorth, A. L., Khanna, N. C., and Firtel, R. A. (1989). A trans-acting factor required for cAMP-induced gene expression in Dictyostelium is regulated developmentally and induced by cAMP. *Genes Dev.* **3**, 747–759.
- Hjorth, A. L., Pears, C., Williams, J. G., and Firtel, R. A. (1990). A developmentally regulated trans-acting factor recognizes dissimilar G/C-rich elements controlling a class of cAMP-inducible Dictyostelium genes. *Genes Dev.* **4**, 419–432.
- Hopper, N. A., Harwood, A. J., Bouzid, S., Veron, M., and Williams, J. G. (1993). Activation of the prespore and spore cell pathway of Dictyostelium differentiation by cAMP-dependent protein kinase and evidence for its upstream regulation by ammonia. *EMBO J.* **12**, 2459–2466.
- Hopper, N. A., Sanders, G. M., Fosnaugh, K. L., Williams, J. G., and Loomis, W. F. (1995). Protein kinase A is a positive regulator of spore coat gene transcription in Dictyostelium. *Differentiation* **58**, 183–188.
- Howard, P. K., Sefton, B. M., and Firtel, R. A. (1992). Analysis of a spatially regulated phosphotyrosine phosphatase identifies tyrosine phosphorylation as a key regulatory pathway in Dictyostelium. *Cell* **71**, 637–647.
- Huang, H. J., Takagawa, D., Weeks, G., and Pears, C. (1997). Cells at the center of Dictyostelium aggregates become spores. *Dev. Biol.* **192**, 564–571.
- Insall, R., Nayler, O., and Kay, R. R. (1992). DIF-1 induces its own breakdown in Dictyostelium. *EMBO J.* **11**, 2849–2854.
- Jain, R., Brazill, D. T., Cardelli, J. A., Bush, J., and Gomer, R. H. (1997). Autocrine factors controlling early development. In “Dictyostelium: A Model System for Cell and Developmental Biology” (Y. Maeda, K. Inouye, and I. Takeuchi, Eds.), pp. 219–234. Universal Academy Press, Tokyo.
- Jain, R., Yuen, I. S., Taphouse, C. R., and Gomer, R. H. (1992). A density-sensing factor controls development in Dictyostelium. *Genes Dev.* **6**, 390–400.
- Jermyn, K., Traynor, D., and Williams, J. (1996). The initiation of basal disc formation in Dictyostelium discoideum is an early event in culmination. *Development* **122**, 753–760.
- Jermyn, K. A., Berks, M., Kay, R. R., and Williams, J. G. (1987). Two distinct classes of prestalk-enriched mRNA sequences in Dictyostelium discoideum. *Development* **100**, 745–755.
- Jermyn, K. A., Duffy, K. T., and Williams, J. G. (1989). A new anatomy of the prestalk zone in Dictyostelium. *Nature* **340**, 144–146.
- Jermyn, K. A., and Williams, J. G. (1991). An analysis of culmination in Dictyostelium using prestalk and stalk-specific cell autonomous markers. *Development* **111**, 779–787.
- Johnson, R. L., Saxe, C. L., III, Gollop, R., Kimmel, A. R., and Devreotes, P. N. (1993). Identification and targeted gene disruption of cAR3, a cAMP receptor subtype expressed during multicellular stages of Dictyostelium development. *Genes Dev.* **7**, 273–282.
- Kawata, T., Early, A., and Williams, J. (1996). Evidence that a combined activator–repressor protein regulates Dictyostelium stalk cell differentiation. *EMBO J.* **15**, 3085–3092.
- Kawata, T., Shevchenko, A., Fukuzawa, M., Jermyn, K. A., Totty, N. F., Zhukovskaya, N. V., Sterling, A. E., Mann, M., and Williams, J. G. (1997). SH2 signaling in a lower eukaryote: A STAT protein that regulates stalk cell differentiation in Dictyostelium. *Cell* **89**, 909–916.
- Kay, R. R. (1997). Dictyostelium development: Lower STATs. *Curr. Biol.* **7**, R723–R725.
- Kay, R. R. (1998). The biosynthesis of differentiation-inducing factor, a chlorinated signal molecule regulating Dictyostelium development. *J. Biol. Chem.* **273**, 2669–2675.
- Kay, R. R., Large, S., Traynor, D., and Nayler, O. (1993). A localized differentiation-inducing-factor sink in the front of the Dictyostelium slug. *Proc. Natl. Acad. Sci. USA* **90**, 487–491.

- Kay, R. R., and Williams, J. G. (1999). The Dictyostelium genome project: An invitation to species hopping. *Trends Genet.* **15**, 294–297.
- Kesbeke, F., Baraniak, J., Bulgakov, R., Jastorff, B., Morr, M., Petridis, G., Stec, W. J., Seela, F., and Van Haastert, P. J. M. (1985). Cyclic nucleotide specificity of the activator and catalytic sites of a cGMP stimulated cGMP phosphodiesterase from Dictyostelium discoideum. *Eur. J. Biochem.* **151**, 179–186.
- Kim, H. J., Chang, W. T., Meima, M., Gross, J. D., and Schaap, P. (1998). A novel adenylyl cyclase detected in rapidly developing mutants of Dictyostelium. *J. Biol. Chem.* **273**, 30859–39862.
- Kimmel, A. R., and Firtel, R. A. (1991). cAMP signal transduction pathways regulating development of Dictyostelium discoideum. *Curr. Opin. Genet. Dev.* **1**, 383–390.
- Klein, C., and Darmon, M. (1976). A differentiation stimulating factor induces cell sensitivity to 3,5'-cyclic AMP pulses in Dictyostelium discoideum. *Proc. Natl. Acad. Sci. USA* **73**, 1250–1254.
- Klein, P. S., Sun, T. J., Saxe, C. L., III, Kimmel, A. R., Johnson, R. L., and Devreotes, P. N. (1988). A chemoattractant receptor controls development in Dictyostelium discoideum. *Science* **241**, 1467–1472.
- Kopachik, W., Oochata, W., Dhokia, B., Brookman, J. J., and Kay, R. R. (1983). Dictyostelium mutants lacking DIF, a putative morphogen. *Cell* **33**, 397–403.
- Kreff, M., Voet, L., Gregg, J. H., Mairhofer, H., and Williams, K. L. (1984). Evidence that positional information is used to establish the prestalk–prespore pattern in Dictyostelium discoideum aggregates. *EMBO J.* **3**, 201–206.
- Kumagai, A., and Okamoto, K. (1986). Prespore-inducing factors in Dictyostelium discoideum. Developmental regulation and partial purification. *Development* **31**, 79–84.
- Kuspa, A., and Loomis, W. F. (1992). Tagging developmental genes in Dictyostelium by restriction enzyme-mediated integration of plasmid DNA. *Proc. Natl. Acad. Sci. USA* **89**, 8803–8807.
- Leach, C. K., Ashworth, J. M., and Garrod, D. R. (1973). Cell sorting out during the differentiation of mixtures of metabolically distinct populations of Dictyostelium discoideum. *J. Embryol. Exp. Morphol.* **29**, 647–661.
- Levine, H., Tsimring, L., and Kessler, D. (1997). Computational modeling of mound development in Dictyostelium. *Physica D* **106**, 375–388.
- Loomis, W. F., Ed. (1982). “The Development of Dictyostelium discoideum.” Academic Press, New York.
- Loomis, W. F. (1993). Lateral inhibition and pattern formation in Dictyostelium. *Curr. Top. Dev. Biol.* **28**, 1–46.
- Loomis, W. F. (1996). Genetic networks that regulate development in Dictyostelium cells. *Microbiol. Rev.* **60**, 135.
- Loomis, W. F. (1998). Role of PKA in the timing of developmental events in Dictyostelium cells. *Microbiol. Mol. Biol. Rev.* **62**, 684.
- Loomis, W. F., and Cann, R. (1982). Bibliography on Dictyostelium. In “The Development of Dictyostelium discoideum” (W. F. Loomis, Ed.), pp. 451–538. Academic Press, New York.
- Loomis, W. F., Kuspa, A., and Shaulsky, G. (1998). Two-component signal transduction systems in eukaryotic microorganisms. *Curr. Opin. Microbiol.* **1**, 643–648.
- Louis, J. M., Ginsburg, G. T., and Kimmel, A. R. (1994). The cAMP receptor CAR4 regulates axial patterning and cellular differentiation during late development of Dictyostelium. *Genes Dev.* **8**, 2086–2096.
- Louis, J. M., Saxe, C. L., III, and Kimmel, A. R. (1993). Two transmembrane signaling mechanisms control expression of the cAMP receptor gene CAR1 during Dictyostelium development. *Proc. Natl. Acad. Sci. USA* **90**, 5969–5973.
- MacWilliams, H., Blaschke, A., and Prause, I. (1985). Two feedback loops may regulate cell-type proportions in Dictyostelium. *Cold Spring Harbor Symp. Quant. Biol.* **50**, 779–785.
- Maeda, Y. (1970). Influence of ionic conditions on cell differentiation and morphogenesis of the cellular slime molds. *Dev. Growth Differ.* **12**, 217–227.
- Maeda, Y. (1993). Pattern formation in a cell-cycle dependent manner during the development of Dictyostelium discoideum. *Dev. Growth Differ.* **35**, 609–616.
- Mann, S. K. O., Brown, J. M., Briscoe, C., Parent, C., Pitt, G., Devreotes, P. N., and Firtel, R. A. (1997). Role of cAMP-dependent protein kinase in controlling aggregation and postaggregative development in Dictyostelium. *Dev. Biol.* **183**, 208–221.
- Mann, S. K. O., Devreotes, P. N., Elliott, S., Jermyn, K., Kuspa, A., Fehheimer, M., Furukawa, R., Parent, C. A., Segall, J., Shaulsky, G., Vardy, P. H., Williams, J., Williams, K. L., and Firtel, R. A. (1994a). Cell biological, molecular genetic, and biochemical methods to examine Dictyostelium. In “Cell Biology: A Laboratory Handbook” (J. E. Celis, Ed.), pp. 412–451. Academic Press, San Diego.
- Mann, S. K. O., and Firtel, R. A. (1991). A developmentally regulated, putative serine/threonine protein kinase is essential for development in Dictyostelium. *Mech. Dev.* **35**, 89–101.
- Mann, S. K. O., and Firtel, R. A. (1993). cAMP-dependent protein kinase differentially regulates prestalk and prespore differentiation during Dictyostelium development. *Development* **119**, 135–146.
- Mann, S. K. O., Richardson, D. L., Lee, S., Kimmel, A. R., and Firtel, R. A. (1994b). Expression of cAMP-dependent protein kinase in prespore cells is sufficient to induce spore cell differentiation in Dictyostelium. *Proc. Natl. Acad. Sci. USA* **91**, 10561–10565.
- McDonald, S. A. (1986). Cell-cycle regulation of center initiation in Dictyostelium discoideum. *Dev. Biol.* **117**, 546–549.
- McDonald, S. A., and Durston, A. J. (1984). The cell cycle and sorting behaviour in Dictyostelium discoideum. *J. Cell Sci.* **66**, 195–204.
- Mee, J. D., Tortolo, D. M., and Coukell, M. B. (1986). Chemotaxis-associated properties of separated prestalk and prespore cells of Dictyostelium discoideum. *Biochem. Cell Biol.* **64**, 722–732.
- Mehdy, M. C., and Firtel, R. A. (1985). A secreted factor and cyclic AMP jointly regulate cell-type-specific gene expression in Dictyostelium discoideum. *Mol. Cell. Biol.* **5**, 705–713.
- Mehdy, M. C., Ratner, D., and Firtel, R. A. (1983). Induction and modulation of cell-type specific gene expression in Dictyostelium. *Cell* **32**, 763–771.
- Meima, M. E., and Schaap, P. (1999). Fingerprinting of adenylyl cyclase activities during Dictyostelium development indicates a dominant role for adenylyl cyclase B in terminal differentiation. *Dev. Biol.* **212**, 182–190.
- Mohanty, S., Jermyn, K. A., Early, A., Kawata, T., Aubry, L., Ceccarelli, A., Schaap, P., Williams, J. G., and Firtel, R. A. (1999). Evidence that the Dictyostelium Dd-STATA protein is a repressor that regulates commitment to stalk cell differentiation and is also required for chemotaxis. *Development* **126**, 3391–3405.
- Morio, T., Urushihara, H., Saito, T., Ugawa, Y., Mizuno, H., Yoshida, M., Yoshino, R., Mitra, B. N., Pi, M., Sato, T., Takeuchi, K., Yasukawa, H., Williams, J., Maeda, M., Takeuchi, I., Ochiai, H., and Tanaka, Y. (1998). The Dictyostelium developmental cDNA project: Generation and analysis of expressed

- sequence tags from the first-finger stage of development. *DNA Res.* **5**, 335–340.
- Ohmori, R., and Maeda, Y. (1987). The developmental fate of Dictyostelium discoideum cells depends greatly on the cell-cycle position at the onset of starvation. *Cell Differ.* **22**, 11–18.
- Oohata, A. A., Nakagawa, M., Tasaka, M., and Fujii, S. (1997). A novel prespore-cell-inducing factor in Dictyostelium discoideum induces cell division of prespore cells. *Development* **124**, 2781–2787.
- Parent, C. A., Blacklock, B. J., Froehlich, W. M., Murphy, D. B., and Devreotes, P. N. (1998). G protein signaling events are activated at the leading edge of chemotactic cells. *Cell* **95**, 81–91.
- Parent, C. A., and Devreotes, P. N. (1996). Molecular genetics of signal transduction in Dictyostelium. *Annu. Rev. Biochem.* **65**, 411–440.
- Pears, C. J., and Williams, J. G. (1988). Multiple copies of a G-rich element upstream of a cAMP-inducible Dictyostelium gene are necessary but not sufficient for efficient gene expression. *Nucleic Acids Res.* **16**, 8467–8486.
- Plyte, S. E., O'Donovan, E., Woodgett, J. R., and Harwood, A. J. (1999). Glycogen synthase kinase-3 (GSK-3) is regulated during Dictyostelium development via the serpentine receptor cAR3. *Development* **126**, 325–333.
- Powell-Coffman, J. A., Schnitzler, G. R., and Firtel, R. A. (1994). A GBF-binding site and a novel AT element define the minimal sequences sufficient to direct prespore-specific expression in Dictyostelium discoideum. *Mol. Cell. Biol.* **14**, 5840–5849.
- Raper, K. B. (1940). Pseudoplasmodium formation and organization in Dictyostelium discoideum. *J. Elisha Mitchell Sci. Soc.* **56**, 241–282.
- Rathi, A., Kayman, S. C., and Clarke, M. (1991). Induction of gene expression in Dictyostelium by prestarvation factor, a factor secreted by growing cells. *Dev. Genet.* **12**, 82–87.
- Rogers, K. C., Ginsburg, G. T., Mu, X., Gollop, R., Balint-Kurti, P., Louis, J. M., and Kimmel, A. R. (1997). The cAMP receptor gene family of Dictyostelium discoideum: Expression, regulation, function. In "Dictyostelium: A Model System for Cell and Developmental Biology" (Y. Maeda, K. Inouye, and I. Takeuchi, Eds.), pp. 163–172. Universal Academy Press, Tokyo.
- Sakai, Y. (1973). Cell type conversion in isolated prestalk and prespore fragments of the cellular slime mold Dictyostelium discoideum. *Dev. Growth Differ.* **15**, 11–19.
- Saxe, C. L., III, Ginsburg, G. T., Louis, J. M., Johnson, R., Devreotes, P. N., and Kimmel, A. R. (1993). CAR2, a prestalk cAMP receptor required for normal tip formation and late development of Dictyostelium discoideum. *Genes Dev.* **7**, 262–272.
- Saxe, C. L., III, Johnson, R. L., Devreotes, P. N., and Kimmel, A. R. (1991). Expression of a cAMP receptor gene of Dictyostelium and evidence for a multigene family. *Genes Dev.* **5**, 1–8.
- Saxe, C. L., III, Yu, Y. M., Jones, C., Bauman, A., and Haynes, C. (1996). The cAMP receptor subtype cAR2 is restricted to a subset of prestalk cells during Dictyostelium development and displays unexpected DIF-1 responsiveness. *Dev. Biol.* **174**, 202–213.
- Schnitzler, G. R., Briscoe, C., Brown, J. M., and Firtel, R. A. (1995). Serpentine cAMP receptors may act through a G protein-independent pathway to induce postaggregative development in Dictyostelium. *Cell* **81**, 737–745.
- Schnitzler, G. R., Fischer, W. H., and Firtel, R. A. (1994). Cloning and characterization of the G-box binding factor, an essential component of the developmental switch between early and late development in Dictyostelium. *Genes Dev.* **8**, 502–514.
- Schulkes, C., and Schaap, P. (1995). cAMP-dependent protein kinase activity is essential for preaggregative gene expression in Dictyostelium. *FEBS Lett.* **368**, 381–384.
- Segall, J. E., Kuspa, A., Shaulsky, G., Ecke, M., Maeda, M., Gaskins, C., and Firtel, R. A. (1995). A MAP kinase necessary for receptor-mediated activation of adenylyl cyclase in Dictyostelium. *J. Cell Biol.* **128**, 405–413.
- Shaulsky, G., Escalante, R., and Loomis, W. F. (1996). Developmental signal transduction pathways uncovered by genetic suppressors. *Proc. Natl. Acad. Sci. USA* **93**, 15260–15265.
- Shaulsky, G., Fuller, D., and Loomis, W. F. (1998). A cAMP-phosphodiesterase controls PKA-dependent differentiation. *Development* **125**, 691–699.
- Shaulsky, G., Kuspa, A., and Loomis, W. F. (1995). A multidrug resistance transporter serine protease gene is required for prestalk specialization in Dictyostelium. *Genes Dev.* **9**, 1111–1122.
- Shaulsky, G., and Loomis, W. F. (1993). Cell type regulation in response to expression of ricin-A in Dictyostelium. *Dev. Biol.* **160**, 85–98.
- Shaulsky, G., and Loomis, W. F. (1996). Initial cell type divergence in Dictyostelium is independent of DIF-1. *Dev. Biol.* **174**, 214–220.
- Siegert, F., and Weijer, C. J. (1992). Three-dimensional scroll waves organize Dictyostelium slugs. *Proc. Natl. Acad. Sci. USA* **89**, 6433–6437.
- Siegert, F., and Weijer, C. J. (1995). Spiral and concentric waves organize multicellular Dictyostelium mounds. *Curr. Biol.* **5**, 937–943.
- Siegfried, E., Chou, T.-B., and Perrimon, N. (1992). Wingless signaling acts through zeste-white 3, the Drosophila homolog of glycogen synthase kinase 3, to regulate engrailed and establish cell fate. *Cell* **71**, 1167–1179.
- Simon, M. N., Pelegrini, O., Veron, M., and Kay, R. R. (1992). Mutation of protein kinase-A causes heterochronic development of Dictyostelium. *Nature* **356**, 171–172.
- Soderbom, F., Anjard, C., Iranfar, N., Fuller, D., and Loomis, W. F. (1999). An adenylyl cyclase that functions during late development of Dictyostelium. *Development* **126**, in press.
- Soderbom, F., and Loomis, W. F. (1998). Cell-cell signaling during Dictyostelium development. *Trends Microbiol.* **6**, 402–406.
- Sternfeld, J. (1979). Evidence for differential cellular adhesion as the mechanism of sorting-out of various cellular slime mold species. *J. Embryol. Exp. Morphol.* **53**, 163–178.
- Sternfeld, J. (1992). A study of pstB cells during Dictyostelium migration and culmination reveals a unidirectional cell type conversion process. *Wilhelm Roux's Arch. Dev. Biol.* **201**, 354–363.
- Sternfeld, J., and David, C. N. (1981). Cell sorting during pattern formation in Dictyostelium. *Differentiation* **20**, 10–21.
- Sternfeld, J., and David, C. N. (1982). Fate and regulation of anterior-like cells in Dictyostelium slugs. *Dev. Biol.* **93**, 111–118.
- Sun, T. J., and Devreotes, P. N. (1991). Gene targeting of the aggregation stage cAMP receptor cAR1 in Dictyostelium. *Genes Dev.* **5**, 572–582.
- Sundaram, M., and Han, M. (1996). Control and integration of cell signaling pathways during C. elegans vulval development. *BioEssays* **18**, 473–480.
- Tasaka, M., and Takeuchi, I. (1981). Role of cell sorting in pattern formation in Dictyostelium discoideum. *Differentiation* **18**, 191–196.

- Thomason, P. A., Traynor, D., Cavet, G., Chang, W. T., Harwood, A. J., and Kay, R. R. (1998). An intersection of the cAMP/PKA and two-component signal transduction systems in Dictyostelium. *EMBO J.* **17**, 2838–2845.
- Town, C., and Gross, J. (1978). The role of cyclic nucleotides and cell agglomeration in postaggregative enzyme synthesis in *Dictyostelium discoideum*. *Dev. Biol.* **63**, 412–420.
- Town, C. D., Gross, J. D., and Kay, R. R. (1976). Cell differentiation without morphogenesis in Dictyostelium discoideum. *Nature* **262**, 717–719.
- Traynor, D., Kessin, R. H., and Williams, J. G. (1992). Chemotactic sorting to cAMP in the multicellular stages of Dictyostelium development. *Proc. Natl. Acad. Sci. USA* **89**, 8303–8307.
- Van Lookeren Campagne, M. M., Wang, M., Spek, W., Peters, D., and Schaap, P. (1988). Lithium respecifies cyclic AMP-induced cell-type specific gene expression in Dictyostelium. *Dev. Genet.* **9**, 589–596.
- Verkerke van Wijk, I., Kim, J. Y., Brandt, R., Devreotes, P. N., and Schaap, P. (1998). Functional promiscuity of gene regulation by serpentine receptors in Dictyostelium discoideum. *Mol. Cell Biol.* **18**, 5744–5749.
- Wang, B., and Kuspa, A. (1997). Dictyostelium development in the absence of cAMP. *Science* **277**, 251–254.
- Wang, M., Aerts, R. J., Spek, W., and Schaap, P. (1988). Cell cycle phase in *Dictyostelium discoideum* is correlated with the expression of cyclic AMP production, detection, and degradation. *Dev. Biol.* **125**, 410–416.
- Wang, N., Soderbom, F., Anjard, C., Shaulsky, G., and Loomis, W. F. (1999). SDF-2 induction of terminal differentiation in Dictyostelium discoideum is mediated by the membrane-spanning sensor kinase DhkA. *Mol. Cell Biol.* **19**, 4750–4756.
- Weijer, C. J., Duschl, G., and David, C. N. (1984a). Dependence of cell-type proportioning and sorting on cell cycle phase in Dictyostelium discoideum. *J. Cell Sci.* **70**, 133–145.
- Weijer, C. J., Duschl, G., and David, C. N. (1984b). A revision of the Dictyostelium discoideum cell cycle. *J. Cell Sci.* **70**, 111–131.
- Williams, J. (1995). Morphogenesis in Dictyostelium: New twists to a not-so-old tale. *Curr. Opin. Genet. Dev.* **5**, 426–431.
- Williams, J. G. (1991). Regulation of cellular differentiation during Dictyostelium morphogenesis. *Curr. Opin. Genet. Dev.* **1**, 358–362.
- Williams, J. G., Duffy, K. T., Lane, D. P., McRobbie, S. J., Harwood, A. J., Traynor, D., Kay, R. R., and Jermyn, K. A. (1989). Origins of the prestalk–prespore pattern in Dictyostelium development. *Cell* **59**, 1157–1163.
- Wood, S. A., Ammann, R. R., Brock, D. A., Li, L., Spann, T., and Gomer, R. H. (1996). RtoA links initial cell type choice to the cell cycle in Dictyostelium. *Development* **122**, 3677–3685.
- Yasukawa, H., Mohanty, S., and Firtel, R. A. (1998). Identification and analysis of a gene that is essential for morphogenesis and prespore cell differentiation in Dictyostelium. *Development* **125**, 2565–2576.
- Zhukovskaya, N., Early, A., Kawata, T., Abe, T., and Williams, J. (1996). cAMP-dependent protein kinase is required for the expression of a gene specifically expressed in Dictyostelium prestalk cells. *Dev. Biol.* **179**, 27–40.

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