MICROBIOLOGICAL REVIEWS, Mar. 1996, p. 135–150 Vol. 60, No. 1
0146-0749/96/$04.00+0

Genetic Networks That Regulate Development in *Dictyostelium* Cells

WILLIAM F. LOOMIS*

Center for Molecular Genetics, Department of Biology, University of California San Diego, La Jolla, California 92093

INTRODUCTION

In 1961, Monod and Jacob (111) pointed out that a regulatory gene that is sensitive to the product of another regulatory gene whose activity it controls could switch to a stable state, either active or inactive, following a transient signal from the environment. They considered several more complicated genetic networks that could account, in principle, for virtually any pattern of differentiation during embryology. However, they did not have available the detailed genetic and biochemical studies necessary to test their proposed mechanisms in differentiating cells. Much has changed in the last 35 years with the advent of molecular genetic techniques, and we are now in a position to recognize the detailed mechanisms and causal connections that regulate the patterns of temporal and cell-type-specific expression of genes involved in the development of a variety of multicellular organisms. The foundations for recognizing the interactions of genes and their protein products came from biochemical studies with purified components. For instance, surface receptors were biochemically shown to interact with trimeric G proteins that transduced the signal via a self-regulating protein kinase cascade (4, 54). Further study has shown that similar networks function in signal transduction pathways found in many tissues and organisms. More recently, genetic techniques have permitted such networks to be perturbed by knocking out specific components or expressing dominant varients of the components. Analyses of the consequences of such mutations have provided direct tests of the proposed regulatory mechanisms. Molecular genetic techniques sufficient for such detailed tests are available for only a small number of eukaryotic multicellular systems, including *Dictyostelium*, *Caenorhabditis*, and *Drosophila* species and, to a limited extent, zebra fish and mice. However, basic cellular processes appear to be conserved to a surprising extent, and understanding the logic and molecular mechanisms of development in one system contributes to understanding similar processes in other organisms, including humans.

Dictyostelium amoebae grow by simple binary division such that segregants, transformants, and mutant clones can be isolated from large populations by microbial genetic techniques (88, 89, 94). When the cells are washed free of nutrients, they rapidly aggregate to form organisms containing up to 10^5 cells and differentiate into four different cell types without further cell division. Within 24 h after the initiation of development, they form the terminal structure, in which a ball of spores is held on a cellular stalk by upper and lower cup cells (76, 93). Although each fruiting body is only 1 mm tall, it is relatively easy to synchronously develop billions of cells to provide sufficient material at every stage for high-resolution biochemical analyses of even minor components (151).

Dictyostelium development can be roughly divided into four stages, which overlap to some extent: (i) aggregation, when the previously solitary cells differentiate to become sociable and form mounds; (ii) postaggregation, when the major cell types, prespore and prestalk cells, diverge; (iii) cell type specialization, when the proportions of various cell types are regulated by intercellular interactions; and (iv) terminal differentiation, when the cell types form either spores or stalk cells and the fruiting body is built. Advances in molecular genetic techniques over the last few years have significantly increased the rate at which new developmental genes are being discovered and characterized (28, 43, 44, 88, 89). Although less than one-fifth of developmental genes have now been characterized, we can begin to see how some of them are connected into networks with properties that exceed their individual roles. This review will consider the signals, both internal and external, that initiate and integrate developmental processes as well as the networks that generate physiologically functional units.

AGGREGATION

The first environmental signal which affects developmental genes is a 68-kDa protein referred to as prestarvation factor or PSF (17, 133). It is synthesized and secreted by all cells even while they are growing but accumulates to the threshold level at which it can be recognized only when the cell density is high. Thus, *Dictyostelium* cells have a way of determining when there are so many cells in a given locale that the likelihood of imminent starvation is high. They respond by expressing a subset of developmental genes, one of which encodes a small surface

* Phone: (619) 534-2543. Fax: (619) 453-0053. Electronic mail address: wloomis@UCSD.edu.

TABLE 1. Aggregation stage genes

Gene	Product	Function	Phenotype of null mutant	Reference
acaA	Adenylyl cyclase	Synthesis of cAMP	Lack of aggregation	128
carA	CAR1	cAMP receptor	Lack of aggregation	150
carC	CAR3	cAMP receptor	None	79
cmfA	80-kDa protein	Mass effector	Lack of aggregation	57
csaA	gp80	Cell-cell adhesion	Small slugs	117
dagA	CRAC	Signal relay	Lack of aggregation	72
erkA	MAP kinase	Protein phosphorylation	Lethal	52
erkB	MAP kinase	Signal relay	Lack of aggregation	143
gpaB	Gα2	Signal relay	Lack of aggregation	87
gpbA	Gβ	Signal relay	Lack of aggregation	160
pdsA	Phosphodiesterase	Breakdown of cAMP	Lack of aggregation	88
pkaA	Protein kinase A	Protein phosphorylation	Lack of aggregation	62
ptpA	PTP1	Protein tyrosine phosphatase		70
ptpB	PTP2	Protein tyrosine phosphatase		71

protein, gp24, that mediates cell-cell adhesion and prepares them for the formation of aggregates (85, 133). When the nutrients are exhausted, cells exit the cell cycle and division stops abruptly (5, 146). Expression of genes necessary for growth, such as those encoding ribosomal proteins, decreases rapidly, whereas other genes become active for the first time (43, 82, 147). Within a few hours, the previously solitary cells are able to signal and respond to each other chemotactically.

Synchronous development can be induced in the laboratory by collecting exponentially growing cells, washing them free of nutrients, and depositing them at high cell density on filters supported over buffered salts (151). Under these conditions, the detailed timing of changes in physiology and macromolecular components can be monitored every hour to determine the order of events. The levels of prestarvation factor and another secreted protein, CMF, rapidly reach threshold levels and result in low levels of transcription of several genes that encode components necessary for production of and chemotactic response to cyclic AMP (cAMP) (16, 18, 55–57, 75, 109, 163). These include the major surface receptor, CAR1, which recognizes extracellular cAMP and transduces the signal into the cell, and the G protein coupled to this receptor. CAR1 is a typical serpentine receptor with seven transmembrane regions and an external high-affinity cAMP-binding site (11, 28, 139, 150). The cytoplasmic face interacts with a trimeric G protein containing the Gα2 subunit and a β subunit. There is only a single gene encoding Gβ in *Dictyostelium* species, and it is essential for development (91, 160). Adenylyl cyclase, the enzyme responsible for cAMP synthesis, is present at a low level, but the level increases dramatically when cells start to respond to exogenous cAMP (96, 128). A gene (*pdsA*) encoding cAMP phosphodiesterase is first transcribed under the control of a vegetative promoter and subsequently expressed when the aggregation-specific promoter is activated by cAMP signalling (42, 129). Another gene (*pdiA*) encodes an inhibitor protein that binds to phosphodiesterase and dramatically reduces its affinity to cAMP (49). This inhibitor gene is active only when the exogenous levels of cAMP are low and is repressed in the presence of high levels of cAMP, when increased phosphodiesterase activity is necessary to keep cAMP levels within bounds. When cAMP binds to CAR1, a pathway is activated that leads to activation of adenylyl cyclase such that the responding cells produce a pulse of cAMP. GDP is exchanged for GTP in the trimeric G protein, which then dissociates into α2 and βγ subunits (78, 148). The βγ subunit is thought to bind an adaptor protein, CRAC, which activates adenylyl cyclase in a process dependent on the mitogen-activated protein (MAP) kinase ERK2 (72, 92, 143). Null mutations in any of these genes result in cells that are unable to aggregate or develop on their own as a result of defects in signal production (Table 1).

There are also G protein-independent responses mediated by cAMP binding to CAR1, which include entry of calcium ions and tyrosine phosphorylation of ERK2 (28, 44, 99). The activity of ERK2 increases to a peak within 30 s and then decays with a half time of about 1 min. The kinetics of activation and deactivation are dependent on CRAC as well as the products of a putative *ras* guanine nucleotide exchange factor, Aimless, and the cAMP-dependent protein kinase, PKA (44a, 99). The pattern of ERK2 activity is similar to that of adenylyl cyclase following a pulse of cAMP, but it is not known at what step in the network of interacting proteins ERK2 may function.

When adenylyl cyclase is activated, it produces a pulse of cAMP, which is immediately secreted, binds to the receptors on adjacent cells, and activates them in turn. cAMP seldom diffuses more than a few cell diameters from the cell that produced it before it is hydrolyzed by the extracellular phosphodiesterase. However, the signal can be propagated over several centimeters by sequential activation of outlying cells. Within 1 min or so after the surface receptor binds cAMP, it becomes phosphorylated and its affinity for cAMP is dramatically reduced (28, 154). Moreover, the signal relay system adapts within 1 min or so such that adenylyl cyclase cannot be further activated until the pathway has returned to its initial state for several minutes. Under normal conditions, cAMP is removed from the environment by the activity of phosphodiesterase and the cells can become resensitized. This activation, desensitization, and resensitization cycle results in the production of cAMP pulses every 5 min or so for several hours. Since cells move chemotactically in response to cAMP pulses, after about 25 pulses they have aggregated into mounds that are several cells thick and the situation changes radically.

A feedback loop connects each pulse of cAMP to the expression of the genes directly involved in the production of subsequent pulses (Fig. 1). This network integrates the levels of each of the components and ensures that all cells will be rapidly induced to express these genes when a few cells start to emit pulses (84). By 6 h of development, most cells have accumulated maximal levels of these "pulse-induced" gene products and are fully responsive to the waves of cAMP that pass over them. They not only relay the signal but also move in the direction of incoming waves and thus aggregate into mounds. The level of cAMP rises significantly within mounds and is no longer pulsatile. Under these conditions, CAR1 is predicted to

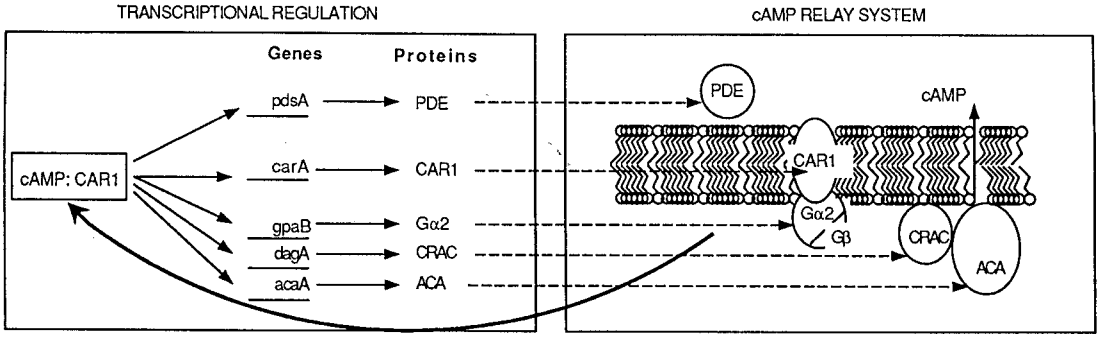

FIG. 1. Aggregation gene network. Genes essential for cAMP response and relay include *pdsA*, which encodes cAMP phosphodiesterase; *carA*, which encodes the major cAMP receptor 1; *gpaB*, which encodes Gα2; *dagA*, which encodes CRAC; and *acaA*, which encodes adenylyl cyclase. The products of these genes form an interacting network in the plasma membrane of aggregating cells. When cAMP binds to CAR1, a pathway is activated that induces these aggregation genes.

remain permanently desensitized and hence no longer responsible for activation of adenylyl cylase. At this time, another cAMP receptor, CAR3, is expressed and coupled to trimeric G proteins containing Gα2 (79, 86, 87). The affinity of CAR3 for cAMP is much lower than that of CAR1, but it does not seem to be subject to the same desensitization process and can take over the role of the surface receptor in the regulation of gene expression under conditions of constant high concentrations of cAMP (74, 149).

The high-affinity cAMP receptor, CAR1, is also coupled to activation of guanylyl cyclase and a poorly understood system of calcium uptake (110, 153). Coupling to guanylyl cyclase is mediated by G proteins containing Gα2, as shown by the lack of activation of guanylyl cyclase or accumulation of cGMP in strains carrying null mutations in the gene encoding Gα2, *gpaB* (86, 121, 122). Likewise, overexpression of *gpaB* leads to increased stimulation of guanylyl cyclase by pulses of cAMP (121). Calcium uptake is stimulated when cAMP binds to CAR1, but this pathway is independent of G proteins, since null mutations in the genes encoding either Gα or Gβ subunits do not affect the process (3, 110). Intercellular calcium ions and cGMP affect many of the processes that appear to be essential for amoeboid movement and chemotactic directionality and thus may mediate the physical aggregation of the cells. Selection of chemically induced mutant strains with impaired chemotactic movement gave several that show normal cAMP relay but reduced activation of guanylyl cyclase (90). The basal level of guanylyl cyclase is unaffected in most of these mutants, and the enzymatic activity is as sensitive to inhibition by calcium ions as in the wild type. The defects must lie in the signal transduction pathway leading to activation of guanylyl cyclase, but the nature of the affected genes is not yet known. Nevertheless, the simultaneous chemotactic and cGMP defects in these mutant strains support the notion that this cyclic nucleotide plays a role in directed ameoboid movement.

Another of the "pulse-induced" genes encodes a protein kinase, PKA, that plays multiple roles in *Dictyostelium* development (62, 63, 68, 69, 102–105). As is the case for all PKA enzymes studied to date, the *Dictyostelium* enzyme has a catalytic subunit that is bound to an inhibitory subunit until the regulatory subunit binds cAMP and dissociates from the complex (119). PKA appears to play a role in the genetic network of aggregation genes, since strains carrying null mutations in *pkaC* do not accumulate adenylyl cyclase and so cannot initiate the feedback loop necessary for high levels of expression of the other genes (102). Such strains fail to aggregate on their own. However, induction of the early genes appears to be largely independent of internal cAMP levels, since strains carrying null mutations in *acaA* or *dagA*, genes that are essential for relay of the pulse, can be induced by artificial nanomolar pulses of exogenous cAMP (72, 128). These results indicate that multiple pathways lead from the cAMP receptor in the plasma membrane to the nucleus, where specific genes are induced.

Starting about 2 h after initiation of development, *csaA* is expressed and its product, the cell-cell adhesion protein gp80, starts to accumulate (117, 118, 120). The rate of accumulation of *csaA* mRNA is stimulated severalfold by pulses of cAMP acting through the surface receptor, such that the rate of gp80 synthesis is maximal by the time cells enter into mounds at about 8 h of development. gp80 accumulates and is inserted into the plasma membrane, where it acts homotypically to mediate EDTA-resistant adhesion. Mutants that fail to accumulate gp80 can aggregate into mounds but are not sufficiently adhesive to be able to generate full-sized slugs and so break up into smaller slugs (41, 80, 117).

Aggregation Gene Network

The feedback loops that Monod and Jacob (111) had in mind when they explored the potential of regulatory networks were derived from processes of transcriptional regulation seen during growth of phage λ and the control of the *lac* operon of *Escherichia coli*. However, these loops were presented in sufficiently general form that they could apply to regulation at many different levels. Those seen in the network of aggregation genes in *Dictyostelium* species are at the level of interactions among the genes of a signal transduction pathway and the pathway itself (Fig. 1). Most of the genes encoding components essential for the relay of cAMP pulses, such as the cAMP receptor and adenylyl cyclase itself, are induced by the interaction of cAMP with the surface receptor. Several other components such as Gβ and ERK2, which are already present at sufficient levels in growing cells, are necessary for this signal transduction pathway. Other components, such as Ras and its guanyl nucleotide exchange factor, Aimless, as well as PKA, appear to be involved in this tightly regulated set of interactions, but they have not been sufficiently characterized at the functional level to be placed in the network in a meaningful way (99, 134, 135).

A network can be considered to be any arrangement of interacting components in which at least one is functionally linked to two or more other components. The nodes in genetic networks are genes, RNAs, proteins, and *cis*-acting regulatory sequences linked by regulatory and physical interactions.

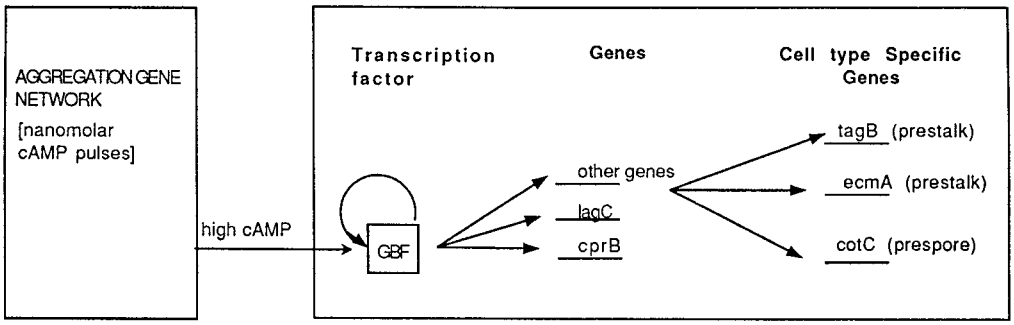

FIG. 2. Postaggregation gene network. When cells have formed mounds, the concentration of cAMP rises to levels at which GBF can stimulate transcription of postaggregative genes. These include *lagC* and *cprB*. A few hours later, the cell-type-specific genes, *tagB*, *ecmA*, and *cotC* are expressed.

When a connection is broken, as when a gene is inactivated, the network may break down or may simply shift to a slightly different state. Often, two or more connections have to be altered before a clearly observable consequence is seen. Strains carrying complex genotypes in which several genes are mutated can be used to test specific models of genetic networks. Such tests are now in their preliminary stages in *Dictyostelium* species.

POSTAGGREGATION

Postaggregative genes, including those that encode the transcription factor G-box-binding factor (GBF), the putative adhesion protein LagC, and a cysteine protease, CP2, are not induced by nanomolar pulses of cAMP but can be induced by micromolar levels of cAMP if the cells had previously developed for 6 h or been pulsed for 6 h in suspension (21–23, 33, 44, 55, 125, 144). GBF is present at low levels during aggregation but reaches maximal levels within 1 h in the presence of 10 mM cAMP. High sustained levels of cAMP lead to adaption of the CAR1-mediated signal transduction pathway, and the levels of mRNA from most of the pulse-induced genes rapidly drop. The gene encoding CAR1 itself is one of the exceptions; it is expressed not only during aggregation but also at later stages, although at lower levels (98). Two different transcriptional initiation sites that are controlled by separate regulatory regions are used for *carA* expression. The early promoter gives rise to a 3.5-kb RNA that is spliced to a 2-kb mRNA, whereas the late promoter gives rise to a 2.6-kb RNA that is spliced to a 2.2-kb mRNA; the alternate splicing does not affect the translated regions, which are predicted to encode exactly the same products. The early promoter is induced by pulses of 20 nM cAMP but repressed by 300 mM cAMP, whereas the late promoter is induced by 300 mM cAMP (98). These independent mechanisms of regulation of *carA* ensure that it is expressed throughout most of the development and suggest that CAR1 functions not only during aggregation but also at subsequent stages. If so, it must be able to function at later stages even when exposed to constant high levels of cAMP.

GBF is a DNA-binding protein that was purified on the basis of its affinity to the regulatory region controlling *cprB*, the gene encoding CP2 (24, 59, 60, 67, 141). It was found to bind to an 8-base sequence referred to as a G-box, which is found upstream of *cprB* as well as in the regulatory regions of other postaggregative genes, including those expressed exclusively in prespore or prestalk cells (12, 13, 40, 48, 123, 124, 126, 131, 161). Accumulation of GBF has been shown to be essential for gene expression during the mound stage, since null mutations in *gbfA*, the gene encoding GBF, fail to express any of the postaggregative genes although mRNAs from pulse-induced genes accumulate normally and their levels decrease normally when mounds are formed (141). Cells of these mutant strains aggregate on time but then disaggregate and reaggregate several hours later, only to disaggregate again, suggesting that they cannot maintain the multicellular state without expressing postaggregative genes. The same gross phenotype is found in strains carrying null mutations in *lagC* (33). These mutants do not accumulate gp80 or display EDTA-resistant adhesion (49a). Since *lagC* is one of the postaggregative genes that is dependent on GBF, the gross phenotype of *gbfA* null mutants may be the consequence of lack of LagC and the EDTA-resistant adhesion mechanism it helps mediate.

The block in development that results from mutational inactivation of *gbfA* can be overcome by expression of the wild-type gene under the control of the vegetative promoter, actin 15 (141, 142). These cells accumulate GBF before the initiation of development but do not express postaggregative genes until the formation of mounds, indicating that although GBF may be essential for postaggregative gene expression, it is not sufficient. However, if cAMP is added a few hours after induction of development in these cells expressing *act15::gbfA*, the postaggregative genes, *lagC* and *cprB*, are immediately induced and their mRNAs reach maximal levels within 1 h (142). The cell-type-specific genes, *ecmA* and *cotC*, are also induced under these conditions, but their mRNAs start to accumulate only several hours after the addition of cAMP, suggesting that they are dependent on expression of intervening genes (Fig. 2).

Cell-Type-Specific Genes

There are three well-characterized prestalk genes, *tagB*, *ecmA*, and *ecmB*, and about six prespore genes, many of which encode the protein components of the spore coats that surround spores (Table 2). Northern (RNA) analyses with probes from these genes have shown that they are not expressed until 10 h of development and remain active throughout the rest of development. Transformed cell lines that carry constructs in which the regulatory regions of either prestalk- or prespore-specific genes control expression of β-galactosidase have been isolated. Staining with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at various stages of development has shown that the first cells expressing these reporter constructs can be observed dispersed throughout mounds. In strains carrying a prestalk-specific reporter construct, the stained cells sort out to the tips that form on the top of the mounds, while in strains carrying prespore-specific reporter constructs, the stained cells are excluded from the tip even when it elongates, falls over, and leads the resulting slug-shaped organism on

TABLE 2. Postaggregation and cell type specialization stage genes

Gene	Product	Function	Phenotype of null mutant	Reference
carB	CAR2	cAMP receptor	Tight aggregates	138
cotA	SP96	Spore coat protein	None	45
cotB	SP70	Spore coat protein	None	46
cotC	SP60	Spore coat protein	None	46
cprB	CP2	Protease	None	157a
ecmA	ST430	Matrix protein	None	113
ecmB	ST310	Matrix protein	None	113
lagC	LagC	Auxilliary adhesion factor	Loose aggregates	33
gbfA	GBF	G-box binding	Loose aggregates	141
pspA	SP29	Matrix protein	None	36
pspB	14-E-6	Spore coat protein	None	130
pspD	PL3	Spore coat protein	None	162
rasD	Ras	Signal transduction	Multiple tips	136
sokA	Transducin	Unknown	Slug	157a
tagB	TagB	PST-A differentiation	Tight aggregates	145
tagC	TagC	PST-A differentiation	Tight aggregates	146
tipA	TipA	Unknown	Multiple tips	149a
ubqB	ubq conj. E3	Protein turnover	Tight aggregates	15a

extended migration. When the fruiting bodies are formed, stalk cells are stained in strains carrying the prestalk-specific construct *ecmA*::*lacZ* and spores are stained in strains carrying the prespore-specific construct *cotB*::*lacZ*. No stained stalk cells could be found in strains carrying *cotB*::*lacZ* and only 2.2% of the spores were stained in the strains carrying *ecmA*::*lacZ* even after 3 days of slug migration (144). These results directly show that there is very little conversion of prestalk and prespore cells even when development is prolonged by extended migration. Therefore, prestalk cells can be stringently defined as those that give rise to stalk cells and express the *ecmA*::*lacZ* reporter construct, and prespore cells can equally be defined as those that express *cotB*::*lacZ* or reporter constructs generated from the other spore coat genes, *cotA*, *cotC*, *pspB*, or *pspD*. The pattern of expression of *lacZ* in strains carrying these reporter constructs has been shown, by using whole-mount in situ hybridization to the respective mRNAs, to accurately represent the expression patterns of the endogenous genes (39). Although X-Gal staining for β-galactosidase activity provides convenient high-resolution analysis at the single-cell level, the regulatory region has to be isolated and fused to *lacZ* before it can be used for staining. In situ hybridization, on the other hand, can directly show the cell type specificity of expression of each new gene as it is discovered.

The observation that a few scattered cells express either *cotB*::*lacZ* or *ecmA*::*lacZ* at the loose mound stage before there is any indication of axial polarization suggests that expression of the cell type markers is independent of position and may be cell autonomous. However, this does not necessarily mean that the choice to become either prespore or prestalk cells is completely random. The population of cells that gives rise to an aggregate is necessarily inhomogeneous: some cells will be larger than others, while some may be more motile. When an exponentially growing culture of cells is induced to initiate development by removing exogenous nutrients and depositing them as a dense layer on a wet surface, some of the cells will have just divided while other will be in late G_2 phase, when they have grown almost enough to divide again. In such cell cycle-unsynchronized populations, cells in early G_2 will preferentially become prestalk cells whereas cells in late G_2 will preferentially become prespore cells (56, 98, 101, 106, 157). Likewise, when large cells that have grown on a rich medium are mixed with smaller cells grown on a more limited medium, the larger cells preferentially become prespore cells (2, 50). However, when pure populations of cells, either ones synchronized in the cell cycle or those with the same nutritional history, are developed, they form prespore and prestalk cells at the normal ratio of 4:1. Clearly, the cells interact and compete for specific cell fates but even slight differences can tip the balance under some conditions.

A few hours after forming a mound, the cells secrete extracellular components including the *ecmA* product, ST450, which form a sheath over the mound and exclude late-arriving cells (107, 108, 114). The relative movement of individual cells in a loose mound appears to be quite limited and random until the sheath is formed, but thereafter prestalk cells move in a spiral pattern towards the top. As this process continues, more and more cells express prestalk markers and sort out to the tip which forms at the top of each mound. Cells expressing prespore markers are left behind in the bulk of the mound. As the tip elongates, almost all of the cells in the posterior express prespore-specific genes. By this stage, there is clear evidence of cell-cell interaction, since the ratio of prespore to prestalk cells at the first finger stage, when the tip is fully elongated, is constant at about 4:1 in slugs that vary by over 10-fold in total cell number (Fig. 3). This size invariance of tissue proportioning is a characteristic of *Dictyostelium* species that is shared by embryos of many organisms and is one of the basic problems that remains to be explained in embryology.

Postaggregative Gene Network

When cells enter into mounds, they are exposed to constant high levels of cAMP and the postaggregation genes become activated. The transcription factor GBF accumulates to low levels during the aggregation phase but requires conditions that are activated by high levels of cAMP to be able to mediate transcription of genes such as *lagC* and *cprB*. When cells are starved in suspension for 6 h and then exposed to high levels of cAMP, *gbfA* mRNA accumulates rapidly to peak levels in a process that appears to depend on the accumulation of GBF itself (141). This autoregulatory circuit functions to rapidly increase the levels of GBF when the conditions permit (Fig. 2). Such a positive-feedback loop can synchronize and gate the transcriptional activity of cells that have entered mounds. *lagC* and *cprB* mRNAs accumulate to maximal levels a few hours later, and the cell-type-specific mRNAs of *ecmA* and *cotC* appear shortly thereafter (141, 142). The role of GBF in this

FIG. 3. Size invariance of cell type proportioning. Whole-mount in situ hybridization with a riboprobe for mRNA of the prespore-specific gene *cotB* was carried out on a population of slugs (39). The proportion of stained (prespore) to unstained (prestalk) cells was not significantly different in slugs which varied by more than 10-fold in size.

developmental pathway is demonstrated by the lack of expression of these genes in mutant strains lacking GBF (Fig. 2). Mutants lacking LagC accumulate *gbfA* and *cprB* mRNAs but are blocked at the loose-aggregate stage and do not express the cell-type-specific mRNAs unless they are developed in mixed aggregates containing wild-type cells (33). Mutants lacking *cprB* develop in an apparently normal manner, indicating that this gene does not play an essential role in morphogenesis under laboratory conditions (119a). The postaggregative gene network appears to be initiated when the signals being received by the cells change from pulses of cAMP in the nanomolar range to constant micromolar levels of cAMP. This leads to the rapid accumulation of GBF and conditions necessary for it to act as a transcriptional activator of genes including *cprB*, *lagC*, and, most probably, other genes that have yet to be recognized. LagC is necessary for the formation of tight mounds surrounded by extracellular matrix material as well as the expression of cell-type-specific genes including *ecmA* and *cotC*. It also appears to be required for the acquisition of EDTA-resistant adhesion and intercellular interactions that can lead to expression of the cell-type-specific genes. Once the extracellular matrix is deposited around mounds, prestalk cells sort out to the top, where they form a tip and become specialized.

CELL TYPE SPECIALIZATION

Several types of prestalk cells can be recognized by the genes and reporter constructs that they express as well as by the roles they play in fruiting-body formation (77). The consequences of mutations in one of the prestalk-specific genes, *tagB*, help to delineate the sequence of events that lead to the specialization of prestalk cell types (145, 146). This gene encodes a protein in which one domain is homologous to the MDR family of ATP-driven transporters and another domain is homologous to the family of serine proteases. These similarities to known peptide transporters suggest that the protein is inserted into cellular membranes, where it transports peptides cleaved from a protein substrate. As might be expected of a mutant lacking an MDR-like protein, several processes of intercellular interaction are defective. Morphogenesis proceeds normally to the tight-mound stage in *tagB* null strains but then arrests with no tips forming on the mounds; neither spores nor stalk cells are formed (145). However, the mutant cells express prespore-specific genes, and when developed in mixed aggregates with wild-type cells, *tagB* null prespore cells encapsulate normally to form spores. On the other hand, *ecmA* is expressed at only 20% of the level seen in wild-type cells when *tagB* null cells are developed on their own. By transforming the reporter construct, *ecmA*::*lacZ*, into *tagB* mutant strains, it is possible to monitor each cell that expresses *ecmA*, even though the level of expression is low. Stained prestalk cells can first be seen at the loose-mound stage and show no sign of spatial localization (143a). After another 8 to 10 h of development, the stained cells are localized at the top of the mound, where they form a cap rather than a tip. Although prestalk cells diverge and sort out from prespore cells in these strains, the *tagB* null prestalk cells cannot fulfill their normal role in slug formation. They appear to be unable to form the anterior structure which leads each slug around as it migrates.

The pattern of expression of *ecmA*::*lacZ* in a genetically wild-type background has delineated two cell types in the prestalk zone of migrating slugs: PST-A and PST-O cells (34, 76). Cells in the anterior 10% of a standing finger or a migrating slug express *ecmA*::*lacZ* at a high level, while cells in the next 10% express the construct at about one-fifth of that level. The most anterior cells enter the stalk tube shortly after it is formed during culmination, while those behind them form the upper cup over the rising sorus and only a few enter the stalk

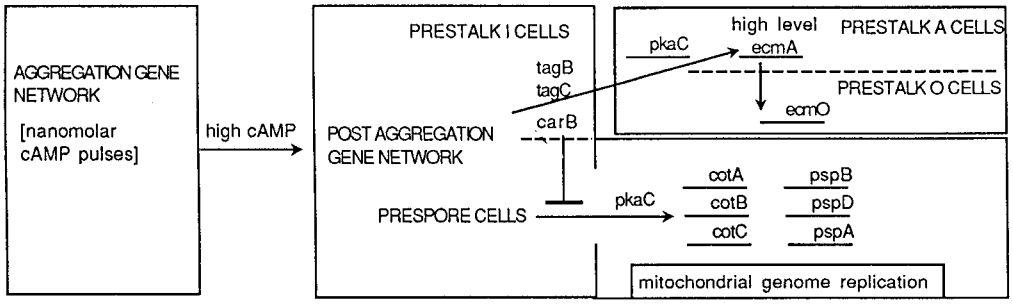

FIG. 4. Cell type specialization. The initial prestalk cells (PST-I) differentiate to PST-A cells, which express *ecmA* at a high level and are the most anterior cells in slugs. Prestalk genes *tagB*, *tagC*, and *carB* are essential for this process. Remaining PST-I cells are induced by PST-A cells to differentiate to PST-O cells, which can be recognized by expression of the *ecmO*::*lacZ* reporter. *carB* also plays a role in limiting the expression of prespore genes. PKA is essential for continued transcription of the spore coat genes *cotA*, *cotB*, and *cotC*. Prespore-specific replication of mitochondrial genomes is independent of aggregation and may influence the initial decision concerning choice of pathway.

when it is almost complete. Cells expressing *ecmA*::*lacZ* at high levels that enter the stalk are defined as PST-A cells, while those expressing the construct at 20% are defined as PST-O cells (76). A subdomain of the *cis*-acting *ecmA* regulatory region has been shown to be sufficient for expression in PST-O cells and to be inactive in PST-A cells (77). Expression of *ecmO*::*lacZ* further defines PST-O cells.

Mutants in which *tagB* is inactivated express the full-length *ecmA* construct *ecmA*::*lacZ* at a low level but do not express *ecmO*::*lacZ* when developed as pure populations (143a). Thus, the stained cells that sort out from prespore cells in *tagB* null mounds are, by definition, not PST-O. Nor are they PST-A cells, since *tagB* mutants are unable to form PST-A cells even when mixed with an excess of wild-type cells, such that they would be expected to receive all the necessary signals. In fact, they are excluded from the most anterior of the slugs that form and do not enter the stalk. The cells in the cap of *tagB* null mounds do not express prespore-specific markers and so are clearly not prespore cells. Therefore, the prestalk cells in the cap have been referred to as PST-I cells, since they appear to be an initial prestalk cell type which can give rise to both PST-A and PST-O cells in wild-type strains (143a). In *tagB* mutant strains, they do not give rise to PST-A cells but can express *ecmO*::*lacZ* when developed in chimeras with wild-type cells. When fruiting bodies are formed from these mixtures of mutant and wild-type cells, the stained mutant cells are found only in the upper cup and not in the stalk, since the *tagB* null PST-I cells cannot differentiate into PST-A cells and so form only PST-O cells.

Northern analyses have shown that *tagB* is expressed at 10 h of development and is localized to prestalk cells (145). Wild-type strains transformed with a construct in which β-galactosidase is driven by the regulatory region of *tagB* give rise to stained cells at the loose-mound stage (146). They arise as dispersed cells that then sort out to the tip, where they differentiate into both PST-A and PST-O cells. During culmination, they form the upper cup and enter the stalk tube soon after it is formed. Transforming the same construct into *tagB* null cells has shown that expression of the reporter gene is not dependent on TagB, as might have been the case if the gene autoregulated. Stained cells arise in a spatially random manner and then sort out to the cap. When these *tagB* null cells carrying the *tagB*::*lacZ* reporter construct are mixed with wild-type cells, the stained *tagB* null cells are found exclusively in the prestalk zone but are excluded from the most anterior of migrating slugs and do not enter the stalk when fruiting bodies are formed (143a, 146). These results show not only that *tagB* is an excellent prestalk specific marker but also that it plays an active role in PST-A differentiation. On the other hand, the inability of *tagB* mutant cells to express *ecmO*::*lacZ* appears to result from the lack of an intercellular signal, since adding wild-type cells to *tagB* mutant cells and developing them in mixed aggregates results in normal expression of this construct. The nature of the signal is not yet known, but considering that its generation is dependent on TagB, it is likely that it is a peptide exported from prestalk cells. The cells responsible for the signal must arise at a later stage than PST-I cells, since these differentiate normally when *tagB* mutant strains are developed but the reporter construct, *ecmO*::*lacZ*, is not expressed in the mutant cells. While it is conceivable that PST-O cells self-stimulate in an autocrine manner, one would then expect them to arise in pure populations of *tagB* null cells; however, this is not seen. It is more likely that PST-A cells produce the signal that induces remaining PST-I cells to differentiate into PST-O cells. In the chimeras in which *tagB* null cells express *ecmO*::*lacZ*, the PST-A cells generated from wild-type cells would produce the signal. Attempts are now being made to characterize the signal and trace its origins.

The other major consequence of mutations in *tagB*, namely, the lack of encapsulation of prespore cells unless wild-type cells are present in mixed aggregates, delineates another intercellular signalling event that is dependent on TagB. However, this process occurs during culmination and is therefore discussed in the next section.

Network That Regulates Cell Type Specialization

Interactions among the cells at the tipped aggregate stage and during slug migration result in differential gene expression in the specialized cell types (Fig. 4). Although we have genetic and physiological evidence concerning some of these processes, we do not yet know the regulatory components that establish the stable transcriptional states (159). It is clear that expression of the prestalk-specific gene, *tagB*, during the post-aggregative stage is a prerequisite for differentiation of PST-A cells (defined as cells which express *ecmA* at high levels, are found at the front of slugs, and enter the stalk tube during culmination). Mutations in *tagB* result in a cell autonomous block to PST-A differentiation, suggesting that the gene product directly activates processes leading to PST-A differentiation or removes a compound that inhibits this process. TagB appears to be a protease fused to an ATP transporter and so may either inactivate an inhibitory protein by proteolysis, remove an inhibitory peptide by export, or both. Mutants lacking

TagB also fail to differentiate into PST-O cells (defined as cells which express the *ecmO*::*lacZ* reporter construct, express the full-length *ecmA*::*lacZ* at about 20% maximal levels, are found just behind PST-A cells in standing fingers and slugs, and enter the stalk tube only at the very end of fruiting-body construction). Differentiation of PST-O cells appears to be a cooperative process, since *tagB* null cells are able to generate this cell type if PST-A cells are present in the same mound. The most parsimonious model to account for the lack of PST-O cells in pure populations of mutant cells is one in which TagB is responsible for transport of a peptide from PST-A cells that actively induces remaining PST-I cells to differentiate along the PST-O pathway (Fig. 4). The peptide signal that is exported in a TagB-dependent manner to permit PST-A differentiation may then induce *ecmO*::*lacZ* in remaining PST-I cells. However, the situation may easily be more complicated, since there is another gene, *tagC*, in which mutations result in exactly the same consequences as mutations in *tagB* (146a). *tagC* is expressed at the same time in development as *tagB* and encodes a protein that is 82% identical to TagB. Thus, TagC may also be a serine protease as well as an ATP-driven transporter. TagB and TagC may function together as a heterodimer; however, overexpression of *tagB* fails to overcome the developmental defects in *tagC* mutants (146a). Therefore, it is conceivable that each of the *tag* genes is responsible for a separate signalling process.

There is both genetic and developmental evidence that PKA plays a role in the same pathway to prestalk specialization as TagB (61, 143a). Since PKA is also essential at an earlier stage in development, it was necessary to find a way to inactivate the enzyme after aggregation was completed. The regulatory region of *ecmA*, which directs transcription of attached genes specifically in prestalk cells, was fused to a modified version of the gene encoding the regulatory subunit of PKA. When this gene is expressed at the mound stage, the dominant negative regulatory protein accumulates and inhibits the catalytic subunit in a manner that cannot be counteracted by the presence of cAMP. This results in the inactivation of PKA specifically in prestalk cells. Cells carrying the *ecmA*::*Rm* construct are arrested as fingerlike structures and form neither spores nor stalk cells, although prespore cells differentiate normally (61). If cells in which PKA is inhibited in prestalk cells are mixed with wild-type cells and allowed to develop together, prespore cells carrying *ecmA*::*Rm* encapsulate into spores; however, when they are mixed with *tagB* null cells, neither strain can form spores (143a). The lack of synergy between two strains which are each able to synergize with wild-type cells indicates that they are defective in the same pathway, which in this case appears to be differentiation of PST-A cells. Like *tagB* null cells, the *ecmA*::*Rm* cells are excluded from the PST-A zone when developed in chimeras with wild-type cells (69a).

PST-I cells also express a prestalk gene, *carB*, that encodes a cAMP receptor, CAR2, with properties somewhat different from those of either CAR1 or CAR3 (138, 140). Mutants in which *carB* is inactivated are detained at the mound stage before tip formation, although some of the aggregates slowly proceed along the morphogenetic pathway to form spores and stalks. Mutations in *carB* also result in overexpression of prespore genes (138), suggesting that the wild-type product normally acts to regulate the level of transcription of these genes in prespore cells (Fig. 4).

While the prespore genes are expressed in a limited number of cells during the postaggregative stage, the number of prespore cells increases significantly during the following few hours. Continued transcription of the spore coat genes during this period depends on PKA (68). The enzyme was specifically inactivated in prespore cells by transforming in a construct, *psaA*::*Rm*, in which the regulatory region of the prespore gene *psaA* drives the gene encoding the modified PKA regulatory subunit (61–63, 68, 69). Strains carrying this construct express the spore coat genes during the mound stage but stop expressing them as soon as tips arise. Since the *psaA* gene is prespore specific, one might expect expression of *psaA*::*Rm* to shut itself off; however, transcription of *psaA* is not PKA dependent, although the stability of its mRNA is dependent on PKA activity (69). Since the stability of the *pkaRm* mRNA is not PKA dependent, the dominant negative subunit continues to accumulate after the tipped aggregate stage in cells carrying *psaA*::*Rm*. Accumulation of the dominant negative regulatory subunit inhibits transcription of many, but not all, prespore-specific genes (Fig. 4). When PKA is inhibited, GBF fails to bind to the G-boxes upstream of the spore coat genes, suggesting that phosphorylation by PKA is essential for this transcription factor to be active (68).

While chromosomal DNA is not replicated during development, mitochondrial DNA is replicated in prespore cells during the mound and first-finger stages (146). It is not yet clear whether cell-type-specific replication of mitochondrial genomes directly affects prespore cell differentiation, but it may account for the higher level of two mitochondrion-specific enzymes, succinic dehydrogenase and cytochrome oxidase, in prespore cells than prestalk cells (152).

As mentioned above, cell type proportions are size invariant by the slug stage, indicating that there is a feedback loop between the number of prespore and prestalk cells that acts continuously following the formation of a tip to ensure that the proper ratio is maintained even if it is perturbed by microsurgical removal of one of the cell types or by cell-type-specific ablation with ricin A (6, 132, 144). While several models can be envisioned to account for size invariance, one of the simpler ones suggests that prespore cells acquire two new properties: (i) they produce an inhibitor of prespore differentiation, and (ii) they become insensitive to this inhibitor (95). If the inhibitor is broken down or inactivated by all cells but made only by prespore cells, a constant proportion of prespore cells will form no matter how many cells happen to be in an aggregate. Likewise, after most of the prespore cells are microsurgically removed or killed by ricin A, the prestalk cells will no longer be exposed to the inhibitor and will convert to prespores until the number of such new prespore cells is sufficient to generate a threshold level of the prespore inhibitor. Whatever the actual proportioning mechanism turns out to be, it is clear that cell type specialization in *Dictyostelium* species results from a series of cooperative and competitive processes that involve cell-cell communication as well as differential gene expression. Although Monod and Jacob (111) were considering networks within single cells, the same reasoning can be applied to processes that connect differentiations in several different cell types.

CULMINATION

The cell types are established during the slug stage but manifest their fates only during culmination, when prespore and prestalk cells undergo terminal differentiation to form spores and stalks, respectively. Several genes have been found (Table 3) that dramatically affect the choice of terminal differentiation, including *gskA* and *dhkA*, which encode protein kinases, and the homeotic gene, *stalky* (*stkA*), which encodes a putative transcription factor (GATA) (60, 115, 116, 156a). Mutants in which *gskA* is inactivated make very few prespore cells and overexpress the prestalk gene *ecmB* (60). *ecmB* en-

TABLE 3. Culmination stage genes

Gene	Product	Function	Phenotype of null mutant	Reference
carD	CAR4	cAMP receptor	Short stalks	97
dhkA	DhkA	Protein kinase	Long stalks; few spores	156a
ecmB	ST310	Stalk matrix	None	113
gpaA	Gα1	G protein	Impaired culmination	29
gpaD	Gα4	G protein	Fingers; no spores	86
gpaG	Gα7	G protein		86
gskA	GSK3	Protein kinase	Few spores	60
spiA	SpiA	Spore coat protein	Impaired spores	137
stkA	Stalky	Putative transcription factor	Very long stalks; no spores	115

codes a large extracellular matrix protein (ST310), which is found predominantly in the stalk tube of fruiting bodies (108, 114). The first cells that express *ecmB* are found dispersed throughout mounds and then sort to the base of tipped mounds, where they are left when the slug migrates away (76). A small set of cells localized in the central core of the prestalk region then express *ecmB* and retain their position at the front of slugs. During culmination, these cells become surrounded by a cellulose-containing tube that initiates stalk formation. Cells expressing *ecmB* during the slug stage are referred to as PST-B cells and are the first cells to vacuolize and become terminally differentiated stalk cells; they are found at the base of the stalk when it is extended to the substratum after pushing through the posterior mass of prespore cells. In *gskA* null strains, the terminal structures are composed mostly of stalk cells, although a few spores are made (60). A much higher proportion of the *gskA* mutant cells express *ecmA* and *ecmB*, and very few express the prespore-specific genes, indicating that the protein kinase GSK3 functions in a pathway that facilitates prespore cell differentiation and inhibits prestalk differentiation. This cell-autonomous phenotype is the opposite of that found in *carD* mutants, which fail to express a prestalk-specific cAMP receptor, CAR4 (97). Therefore, we should consider the possibility that cAMP acts through the CAR4 surface receptor to inhibit GSK3. *carD* mutant strains overexpress prespore genes, including *cotB*, and express *ecmB* at greatly reduced levels (97). Since *ecmB* is repressed and the prespore genes are induced by GSK3, the most direct pathway for the effects of CAR4 is through GSK3 (Fig. 5). Since this serine/threonine kinase is phosphorylated on a tyrosine in the active site, we might expect tyrosine kinases and tyrosine phosphatases to participate in this pathway (70, 71). GSK3 is a member of the glycogen synthetase kinase family that includes *shaggy*, which acts downstream of *wingless* to establish segement polarity in *Drosophila melanogaster* (7, 27, 30). It will be interesting to see if *gskA* is linked into a similar network in *Dictyostelium* species.

dhkA encodes a protein kinase that is homologous to the two-component systems known to mediate responses to extracellular stimuli in bacterial, yeast, and plant cells. In these organisms, an external signal stimulates autophosphorylation of a histidine in one of the components, which then transfers the phosphate to an aspartate on another protein, the response

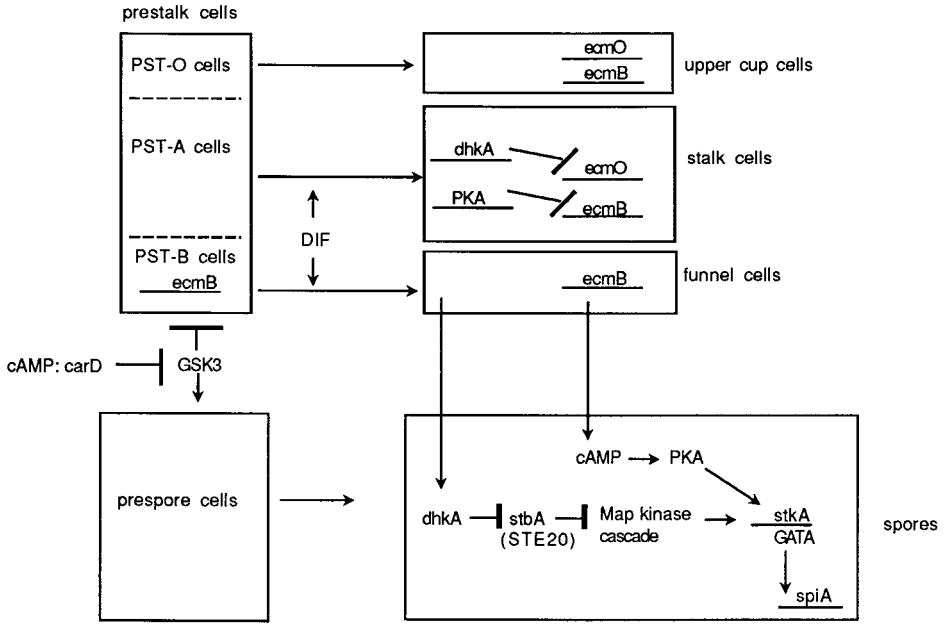

FIG. 5. Terminal differentiation. After 18 h of development, PST-B cells that can be recognized by expression of the *ecmB*::*lacZ* reporter enter the newly formed stalk tube and decend through the mass of cells until they reach the supporting substratum. Expression of *ecmB*::*lacZ* is inhibited in PST-A cells by PKA until they enter the stalk tube. Expression of *ecmO*::*lacZ* is inhibited in PST-A cells by the histidine kinase, DhkA. PST-O cells give rise to the upper cup, where they express both *ecmO*::*lacZ* and *ecmB*::*lacZ*. The chlorinated hexanophenone, DIF, induces terminal differentiation of stalk cells. Prespore cells are induced to express *spiA* and encapsulate by prestalk cells that lie above them. The signal is transduced by a pathway that includes DhkA and PKA. The stalky gene, *stkA*, plays an essential role in spore formation; in its absence, prespore cells differentiate into prestalk cells.

regulator. In bacterial nitrogen metabolism, the response regulator is a transcription factor, while in chemotaxis it controls flagellar movement. In *Dictyostelium* species, *dhkA* plays roles in both prestalk and prespore cells during culmination. Mutants in which this gene is inactivated express *ecmO* in PST-A cells before and after they enter the stalk tube, and the stalks are weakened (156a). More dramatically, very few prespore cells encapsulate into spores. Thus, *dhkA* appears to repress *ecmO* in PST-A cells and to induce encapsulation in prespore cells.

Encapsulation is also affected by PKA, since strains in which the gene encoding the regulatory subunit is inactivated by mutations sporulate precociously. Unlike the situation in wild-type strains, encapsulation in these rapidly developing strains is not dependent on the proximity of prespore cells and prestalk cells, since *pkaR* mutant cells that are dissociated from slugs and suspended in buffer make spores efficiently (95). In wild-type strains, this situation can be mimicked by adding the membrane-permeable derivative of cAMP, 8-Br-cAMP, to the buffer so as to activate PKA. Under these conditions, the late prespore gene, *spiA*, is induced and encapsulation ensues (137). Normally, *spiA* is first expressed in the cells that are closest to prestalk cells at the top of the sorus and then expressed in progressively more distant prespore cells (137). This temporal wave of expression suggests that a diffusible signal triggering *spiA* expression emanates from prestalk cells. The signal may originate in either PST-A or PST-O cells but clearly is not made in PST-I cells, since prespore cells fail to encapsulate in *tagB* mutant strains (143a). The signal may be transduced through either the DhkA pathway or the PKA signal transduction pathway, which appear to be independent since 8-Br-cAMP is still able to induce encapsulation in *dhkA* mutant cells (156a). The pathways appear to converge at a later step leading to encapsulation which may involve the activation of a sporulation-specific transcription factor (Fig. 5).

A series of homeotic mutants in which prespore cells make stalk cells rather than spores have been isolated (115). The resulting fruiting bodies have long, thin stalks and no sori. Even when developed together with wild-type cells, the mutant cells fail to form spores. Seven independent mutations were mapped to the same locus on chromosome 2, and the gene was named *stalky* (*stkA*) (116). It is likely that there is only a single locus which can mutate to give the *stalky* phenotype. The gene has been disrupted by plasmid insertion, cloned, and sequenced (14a). *stkA* encodes a protein with convincing homology to the zinc finger domain of the GATA family of transcriptional regulatory proteins, suggesting that Stalky may be a DNA-binding protein that regulates transcription. Not only do *stkA* strains not encapsulate, but they also fail to express the sporulation marker gene *spiA* consistent with Stalky playing a transcriptional regulatory role. Spore differentiation is completely dependent on Stalky activity in a cell-autonomous manner, and in its absence, prespore cells convert to the stalk cell differentiation pathway, thereby undergoing a homeotic transition.

While the products of the prestalk genes, *ecmA* and *ecmB*, ST430 and ST310, respectively, are not essential for prestalk or stalk differentiation, the genes provide excellent markers for the steps leading to terminal differentiation (12, 34, 113). The *cis*-acting sequences that control transcription in various cell types at various stages are found in the 2-kb regions upstream of these genes. A 161-bp region near the transcriptional start site of the *ecmA* gene is essential for expression in PST-A cells; in its absence, transcription is restricted to PST-O cells. However, if a reporter construct driven by such a deleted regulatory region is present in a strain in which *dhkA* has been inactivated, the reporter gene is expressed in PST-O cells only during the slug stage but is expressed in both PST-O and PST-A cells during culmination (156a). Thus, it appears that the histidine kinase encoded by *dhkA* plays a role in PST-A cells as well as in prespore cells. In wild-type PST-A cells, it acts on the *ecmO* *cis*-acting sites to repress transcription during culmination. In mutants in which *dhkA* is inactivated, there are stained cells extending to the top of partially completed fruiting bodies and the cells in the stalk tube are all stained. In wild-type strains carrying *ecmO*::*lacZ*, cells at the tip are stained only at the end of culmination when the fruiting body is completed.

ecmB is expressed in cells found in the central core of the prestalk region at the anterior of slugs, and it is these cells that lead the progression of the stalk tube down through the mass of prespore cells to the substratum. When a fruiting body is fully formed, these cells are found at the base of the stalks. PST-A cells, which enter the stalk tube just after the PST-B cells, do not express *ecmB* in the slug stage but start to express this gene when they enter the stalk tube. As a result, cells all along the stalk are stained in strains carrying *ecmB*::*lacZ*. Cells in the upper cup, which is formed from anterior-like cells during culmination, are also stained in strains carrying *ecmB*::*lacZ*. Expression in these cells is dependent on a 737-bp region that is found about 1 kb upstream of the transcriptional start site of *ecmB* (12). A 278-bp region proximal to the transcriptional start site of *ecmB* plays an essential role in repression of the gene in PST-A cells while they are still outside the stalk tube. Repression in PST-A cells during the slug stage and prior to terminal differentiation into stalk cells is mediated by a regulatory mechanism functioning through this *cis*-acting region that involves PKA (1, 62, 63). The activity of PKA and the expression of *pkaA* both drop precipitously when cells enter the top of the stalk tube and repression of *ecmB* is relieved (104, 155).

Both *ecmA* and *ecmB* can be induced in cells dissociated soon after they have reached the postaggregation stage and suspended in buffer containing cAMP if the chlorinated hexanophenone DIF-1 is added to 100 nM (1, 14, 158). In the absence of added DIF-1, *ecmA* is expressed at a low level but *ecmB* is not expressed at all. DIF-1 is synthesized by *Dictyostelium* cells following aggregation and accumulates during the slug stage to reach peak levels during culmination. This small, lipid-soluble molecule was purified by a bioassay in which dispersed cells were induced to vacuolize into separate stalk cells and has therefore been referred to as a morphogen (112, 158). However, there is some question whether DIF acts in a concentration-dependent manner to establish the cell types during the postaggregation stage. A small number of cells can be seen at the loose-mound stage that express constructs such as *cotB*::*lacZ* and *ecmA*::*lacZ*, which define prespore and prestalk cells, and yet DIF cannot be measured at that stage. Moreover, these reporter constructs are expressed in *tagB* mutant cells that arrest at the mound stage and never accumulate measurable DIF-1 (143a). While it is conceivable that the DIF-1 threshold for induction is below the DIF-1 threshold for measurement, this supposition is presently untestable. Furthermore, neither of the prestalk-specific genes, *carB* or *tagB*, require DIF-1 for maximal expression (140, 145). At later stages, DIF-1 accumulates to more than 100 nM and may be responsible for maximal induction of *ecmA* and *ecmB* (9). The rate of synthesis of ST430 and ST310, the products of the *ecmA* and *ecmB* genes, respectively, increases dramatically during culmination (114); perhaps as a consequence of DIF-1 release from a sequestered reserve. Addition of weak acids has the same effect as addition of DIF-1, and weak acids are predicted to favor release of DIF-1 from lipid compartments such as inter-

nal membranes into the cytoplasm (8, 58, 156). Addition of weak bases, such as NH_4^+, would have the opposite effect, and NH_4^+ has been shown to delay terminal differentiation of stalk cells, especially in slugger mutants that migrate for extended periods (8, 25, 53, 58).

Overhead light is a strong stimulus for culmination. Within 2 min of overhead illumination 1,2-diacylglycerol rises from basal levels to reach peak levels of 10 mM (20). Phospholipase D, rather than the more common phospholipase C, may be responsible for producing second message, since it has been found that phospholipase C null mutants develop normally (32). The signal transduction pathway by which light affects cellular properties is not yet known but may involve Gα1, since the increase in 1,2-diacylglycerol level is reduced in strains lacking Gα1 and culmination is seriously perturbed (10, 29).

Network That Regulates Culmination

Orchestrating culmination to result in proper terminal differentiation of each of the four cell types in concert requires interaction between prespore and prestalk cells as well as a way to monitor actual steps in morphogenesis, such as entry into the stalk tube. The regulatory network is tied together by signals from the environment eliciting signals from specific cell types that gate differentiations in the entire structure. When a slug migrates to the top of the soil, where these organisms are found, it is exposed to overhead light. This signal is transduced into the cell by a pathway that may be G protein-coupled and results in production of a second message, 1,2-diacylglycerol, which is known to affect various activities including those of some protein kinases. Other kinases, such as PKA, are activated in prespore cells and inhibited in some of the prestalk cells (104). As a result, the pattern of gene expression changes rapidly during culmination. *spiA* is expressed in prespore cells and leads to the accumulation of a protein that lines the inside of the spore coat (136). *ecmB* is expressed in the upper-cup cells as well as in PST-A cells when they enter the stalk tube as a result of relieving the repression mediated by a pathway that includes PKA (35). The PST-O-specific enhancer of *ecmA* continues to function in PST-O cells but can be expressed in PST-A cells unless inhibited by a pathway that includes *dhkA* (156a). *dhkA* is also expressed in prespore cells and plays an essential role in triggering encapsulation in a pathway that also depends on PKA.

The protein kinase encoded by *dhkA* appears to be a member of the family of two-component systems composed of a sensor histidine kinase that responds to extracellular stimuli by autophosphorylation and a response regulator that accepts the phosphate on an aspartate moiety. There are over 40 such systems in bacteria that mediate a variety of responses. In yeasts, the only known sensor, SLN1, inhibits the response regulator, SSK1 (19, 65). Under conditions of high osmolarity, SLN1 is inhibited and SSK1 activates two protein kinases, SSK2 and SSK22, at the top of a MAP kinase cascade that includes the MAP kinase PBS2 and the MAP kinase HOG1 (26, 38, 100). The two-component system that mediates responses to ethylene in *Arabidopsis* species is also linked to a MAP kinase cascade (15, 83), and so it seems worth considering that *dhkA* might also be linked to a MAP kinase cascade. Two MAP kinases have been characterized in *Dictyostelium* species, and there appear to be more to be discovered (44a, 52, 143). ERK1 is a vital gene, precluding the study of null cells, but ERK2 is dispensable for growth (52, 143). This MAP kinase is rapidly activated when phosphorylated on a tyrosine moiety and can phosphorylate serine and threonine groups in the assay substrate myelin basic protein (100, 143). Its normal substrate(s) is unknown. Null mutants lacking ERK2 are able to respond chemotactically to cAMP but are unable to relay the signal, indicating that at least one of the functions of this protein kinase takes place on the cytoplasmic face of the surface membrane. By generating a temperature-sensitive variant of ERK2, it has recently been possible to show that ERK2 plays a distinct role following aggregation (51). When *erkB*(Ts) mutant strains are shifted to the nonpermissive temperature (27°C) following aggregation, prespore-specific mRNAs disappear and aberrant towers lacking spores are made. These results indicate that this MAP kinase may play a role during culmination that is distinct from its role in aggregation (Fig. 5). We have recently isolated a gene, *stbA*, which encodes a member of the STE20 protein kinase family; null mutation in *stbA* suppress the block to sporulation in *tagB* and *tagC* mutants (146a). This phenotype suggests that *stbA* plays an inhibitory role in the pathway to terminal differentiation of spores (Fig. 5). It will be interesting to see if the STE20/MAP kinase cascade that functions during mating and sporulation in yeasts has been conserved in *Dictyostelium* sporulation.

Fruiting bodies formed from wild-type cells have long, gently tapering stalks holding up a ball of spores that gradually turns yellow because of the accumulation of a ζ-carotinoid (93). Construction of a well-proportioned fruiting body appears to depend on a considerable number of genes, since a large number of independent mutant strains with misshapen, aberrant forms have been found. *carD* mutants make fruiting bodies with short, twisted stalks holding up small sori, whereas *gskA* mutants form irregular masses of cells from which a weak stalk protrudes (60). In the absence of the GSK3 protein kinase, most of the prespore cells express *ecmB* and appear to function as PST-B cells. In wild-type strains, GSK3 limits the number of PST-B cells to a small percentage of the total cells in a slug, and these form the anterior funnel which initiates stalk tube formation during culmination. When the population of PST-B cells expands dramatically, as in *gskA* strains, establishment of a functional stalk tube is difficult at best and most of the cells vacuolize and differentiate in place, producing the mound of stalk cells that characterizes these strains. The activity of GSK3 appears to be modulated by extracellular cAMP acting through the surface cAMP receptors encoded by *carD* and, perhaps, also those encoded by *carB* (97, 138). Mutants lacking either CAR2 or CAR4 not only express *ecmB* at reduced levels but also express prespore genes at elevated levels. Since both *carB* and *carD* are prestalk-specific genes expressed in prestalk cells but not in prespore cells, they must be at least partially responsible for the production of a signal that passes from prestalk cells to prespore cells.

The chlorinated hexanophenone DIF-1, which accumulates during the slug stage, is kept at a slightly lower level in the prestalk region than in the prespore region because of the localization of DIF-1 monodechlorinase in prestalk cells (9, 73, 81). This DIF-1-metabolizing enzyme is induced by DIF-1 itself in a feedback loop and is activated by various treatments that disrupt the integrity of slugs (81). Although DIF-1 can repress prespore gene expression in prespore cells when they are dissociated and suspended in buffer containing cAMP and calcium ions, DIF-1 appears to have little effect in the prespore region of intact slugs even though it is present at high concentrations (37, 47). It seems worth considering that most of the DIF-1 might be sequestered in an inactive form during the slug stage and then released during culmination, perhaps as a consequence of changes in the internal pH. During culmination, expression of the DIF-inducible gene, *ecmB*, increases significantly in prestalk cells, which may indicate the release of DIF-1.

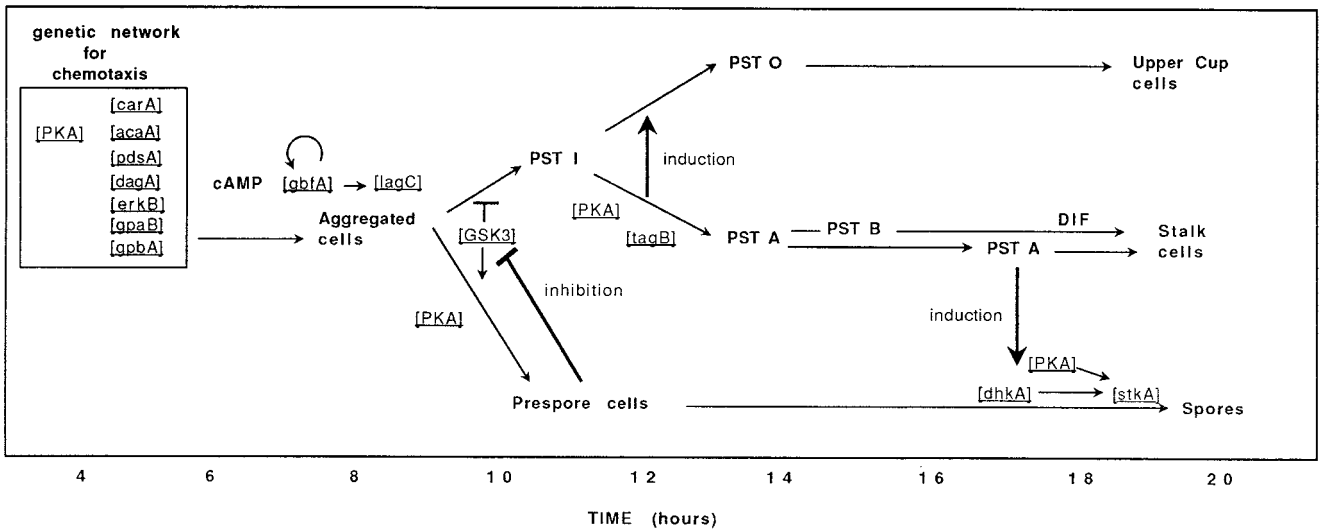

FIG. 6. Network of cell type interactions. The genetic network that is responsible for cellular responses to cAMP, including chemotaxis and relay, leads to the expression of postaggregation genes mediated by GBF. A positive-feedback loop in which GBF induces its own structural gene, *gbfA*, amplifies transcriptional potential during this stage. PKA plays essential roles throughout development, including the aggregation stage, prespore and prestalk A differentiation, and encapsulation. The regulation of cell type proportions may be mediated by an inhibitor, produced by prespore cells, which controls the activity of the protein kinase GSK3. PST-A cells appear to be responsible for induction of PST-O cell differentiation in tipped aggregates and encapsulation of prespore cells in culminants. This scheme may serve as a framework to position the roles of new developmental genes as they are discovered.

NETWORKS OF NETWORKS

Like all complex organisms, the genus *Dictyostelium* evolved in stages. Asocial amoebae just encapsulate in place; other extant species aggregate before encapsulating but do not raise the spores on stalks. Spores can survive high temperatures and desiccation and can even pass unharmed through the gut of a small worm (82a). An aggregate surrounded by an extracellular matrix is protected from larger worms. Lifting the spores on a strong stalk increases the chances of dispersal to new environments. A genetic network permiting encapsulation most probably evolved first and then was incorporated into a larger network that provided the advantages of cell type specialization. Generating a system that ensures size-invariant cell type proportions and the ability to regulate would bring the level of complexity to that now seen in *Dictyostelium* species.

The initial cell-autonomous differentiation of prespore and prestalk cells is reminiscent of the establishment of metazoan embryonic axes by maternal factors. Some gene products that are synthesized during oogenesis in the mother are localized in the eggs; following fertilization, they determine the specialization of cells along the anterior/posterior or dorsal/ventral axes. In this way, the molecular history of the egg establishes future patterns of differentiation. *Dictyostelium* cells do not develop from a fertilized egg, but the cells in an aggregate have histories of growth and division that can distinguish one from another. Even bacterial cells have histories that can affect their fates. One of the paradigms that led Monod and Jacob (111) to explore the potential of genetic networks was the induction of the *lac* operon in *E. coli*. In this system, a bacterial cell that had previously grown on lactose can subsequently grow on lower levels of lactose than a naive cell as a result of the accumulation of lactose permease during the prior period of growth. If a period of growth in the absence of lactose intervenes, some of the cells, but not others, can immediately use lactose as a carbon source even when it is present at very low levels. Those that grow happened to inherit the last few molecules of permease and can take up the sugar and initiate a positive-feedback loop in which lactose induces more permease as well as β-galactosidase. The rest of the cells starve. When a population of *Dictyostelium* cells is induced to develop, some have just divided whereas others are about to divide, and so they will vary with respect to size, energy stores, and the ratio of cytoplasm to nucleus. These and other differences can then form the basis for future choice of pathways. As the number of cells differentiating into prespore or prestalk cells increases, intercellular regulatory mechanisms come into play to establish size-invariant proportions and sorting out of the prestalk cells to the anterior. Prespore cells may secrete a signal to which they have become insensitive that precludes other cells from following the prespore pathway. The proportion of prespore cells will be independent of the total number of cells in such a field and can be easily regulated when perturbed.

The aggregation genetic network that is put in place shortly after the initiation of development allows the cells to cooperate and bring in outlying cells. Many of the components and their connections have been retained almost unchanged in metazoan embryogenesis. The initial excitation of the chemotactic response is mediated by a serpentine, transmembrane seven-helix receptor that is closely related to a wide variety of receptors of neurotransmitters including serotonin, dopamine, acetylcholine, odorants, and light. When CAR1 binds cAMP, it activates heterotrimeric G proteins, catalyzing the exchange of GTP for GDP on the α subunit and the dissociation of the α subunit from the βγ subunit, just as in other G-protein-linked receptors. Both the α and the βγ subunits modulate downstream effectors much as in mammalian cells (28, 54). The conservation of this network attests to its effectiveness in cell-cell communication. The feedback loops that connect receptor excitation to induction of the genes responsible for the components themselves tie the network together and lead directly to the postaggregation network. The relative timing, as well as putative causal relationships of the various developmental genes and the extracellular effectors, is presented as a working model in Fig. 6.

One of the genes that is activated during the aggregation stage encodes a transcription factor, GBF, which is necessary

but not sufficient for expression of the genes which characterize the postaggregation stage. Chemotaxis in response to nanomolar pulses of cAMP is replaced by an environment of constant higher levels of cAMP when the cells pile up in a mound. This environmental change is essential for expression of the postaggregation genes including *lagC*, which is required for signaling involved in the initial establishment of prespore and prestalk cell populations. The relative proportions of these cell types is dependent on a pathway that includes GSK3. When the proper proportion of prespore cells is reached, an inhibitor of further prespore differentiation keeps other cells from following this pathway, and they differentiate as prestalk cells. GSK3 may function in this inhibitory feedback loop (Fig. 6). In *Xenopus* embryos, GSK3 affects dorsoventral axis formation (31, 64, 127). When GSK3 is inactivated in vegetal blastomeres at the eight-cell stage, a secondary axis is formed. These and other results indicate that GSK3 inhibits dorsal differentiation, including neutralization. Perhaps differentiation of the initial prestalk cells should be thought of in relationship to the formation of dorsal somatic tissue in vertebrates.

The initial prestalk cells further differentiate to PST-A cells in a process dependent on the activity of PKA and TagB. PST-A cells then induce remaining PST-I cells to differentiate to PST-O cells and thereby establish the proportions of these cell types (Fig. 6). Continued intercellular communication between the cell types regulates both differentiation and dedifferentiation to maintain optimal proportions of each of the cell types during the slug stage that can be extended for days under conditions where the slugs are migrating phototactically to the surface of the soil.

Culmination is induced by overhead light that might be encountered when a slug reaches the top of the soil. PST-B cells in the core of the prestalk region start to lay down extracellular cellulose fibers to form the mouth of the stalk tube. As the PST-B cells descend through the prespore cells toward the substratum, they are followed by PST-A cells, which start to express *ecmB* as soon as they enter the top of the stalk tube. Before these prestalk cells enter the tube and terminally differentiate, they produce a signal that induces encapsulation in prespore cells. The signal is transduced by protein kinases and transcription factors such that sporulation genes are activated and encapsulation ensues. If the signal is not given or the prespore cells fail to respond to it, as is seen in the *stkA* mutant strains that lack the putative GATA factor, prespore cells will vacuolize and differentiate into stalk cells.

Partly as the result of the relative simplicity and speed with which *Dictyostelium* cells develop, it is possible to take a broad overview that encompasses the whole life cycle. Just as no gene acts alone, no network is independent of the networks that precede it. While our knowledge of the components and connections in the individual networks is still sketchy, the general layout can serve as a starting framework to position the roles of new genes as they are discovered. Techniques such as restriction enzyme-mediated integration allow genes to be recognized on the basis of phenotype alone and then rapidly cloned and sequenced (88). Saturation screens via restriction enzyme-mediated integration are unbiased by preconceptions and so will certainly add new and surprising genes to the networks. This technique can also be used to isolate second-site suppressers of specific developmental mutations (146a). These newly tagged genes will arrive with strong evidence for direct interactions with previously characterized genes and so build the case for complex networks.

ACKNOWLEDGMENTS

I am grateful to Adam Kuspa and Gad Shaulsky for challenging discussions and to many of my colleagues for pertinent data. I thank Ricardo Escalante for the in situ staining of prespore cells in different-sized slugs. I also thank Ricardo Escalante, Richard Firtel, Nir Osherov, and Gad Shaulsky for comments on the manuscript.

This work was supported by NIH grant HD30892.

REFERENCES

1. **Berks, M., and R. R. Kay.** 1990. Combinatorial control of cell differentiation by cAMP and DIF-1 during development of *Dictyostelium discoideum*. Development **110:**977–984.
2. **Blaschke, A., C. Weijer, and H. MacWilliams.** 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.
3. **Blumberg, D. D., J. F. Comer, and K. G. Higinbotham.** 1988. A Ca^{++}-dependent signal transduction system participates in coupling expression of some cAMP-dependent prespore genes to the cell surface receptor. Dev. Genet. **9:**359–370.
4. **Blumer, K., and G. Johnson.** 1994. Diversity in function and regulation of MAP kinase pathways. Trends Genet. **19:**236–240.
5. **Bonner, J. T.** 1960. Development in the cellular slime molds: the role of cell division, cell size, and cell number, p. 1–19. *In* D. Rudnick (ed.), Developing cell systems and their control. Ronald Press, New York.
6. **Bonner, J. T., and M. K. Slifkin.** 1949. A study of the control of differentiation: the proportions of stalk and spore cells in the slime mold *Dictyostelium discoideum*. Am. J. Bot. **36:**727–734.
7. **Bourouis, M., P. Moore, L. Ruel, Y. Grau, P. Heitzler, and P. Simpson.** 1990. An early embryonic product of the gene shaggy encodes a serine/threonine protein kinase related to CDC28/cdc2 subfamily. EMBO J. **9:** 2877–2884.
8. **Bradbury, J. M., and J. D. Gross.** 1989. The effect of ammonia on cell-type-specific enzyme accumulation in *Dictyostelium discoideum*. Cell Differ. Dev. **27:**121–128.
9. **Brookman, J. J., C. D. Town, K. A. Jermyn, and R. R. Kay.** 1982. Developmental regulation of stalk cell differentiation-inducing factor in *Dictyostelium discoideum*. Dev. Biol. **91:**191–196.
10. **Carrel, F., S. Dharmawardhane, A. Clark, J. Powell-Coffman, and R. Firtel.** 1994. Spatial and temporal expression of the *Dictyostelium discoideum* Gα protein subunit G-alpha-2: expression of a dominant negative protein inhibits proper prestalk to stalk differentiation. Mol. Biol. Cell **5:**7–16.
11. **Caterina, M. J., J. L. S. Milne, and P. N. Devreotes.** 1994. Mutation of the third intracellular loop of the cAMP receptor, CAR1, of *Dictyostelium* yields mutants impaired in multiple signaling pathways. J. Biol. Chem. **269:** 1523–1532.
12. **Ceccarelli, A., H. Mahbubani, and J. G. Williams.** 1991. Positively and negatively acting signals regulating stalk cell and anterior-like cell differentiation in *Dictyostelium*. Cell **65:**983–989.
13. **Ceccarelli, A., H. J. Mahbubani, R. Insall, G. Schnitzler, R. A. Firtel, and J. G. Williams.** 1992. A G-rich sequence element common to *Dictyostelium* genes which differ radically in their patterns of expression. Dev. Biol. **152:** 188–193.
14. **Ceccarelli, A., S. J. McRobbie, K. A. Jermyn, K. Duffy, A. Early, and J. G. Williams.** 1987. Structural and functional characterization of a *Dictyostelium* gene encoding a DIF inducible, prestalk-enriched mRNA sequence. Nucleic Acids Res. **15:**7463–7476.

14a. **Chang, C., P. Newell, and J. D. Gross.** Personal communication.

15. **Chen, Q. G., and A. B. Bleecker.** 1995. Analysis of ethylene signal-transduction kinetics associated with seedling-growth response and chitinase induction in wild-type and mutant *Arabidopsis*. Plant Physiol. **108:**597–607.

15a. **Clarke, A., and R. Firtel.** Personal communication.

16. **Clarke, M., N. Dominguez, I. S. Yuen, and R. H. Gomer.** 1992. Growing and starving *Dictyostelium* cells produce distinct density-sensing factors. Dev. Biol. **152:**403–406.
17. **Clarke, M., S. C. Kayman, and K. Riley.** 1987. Density-dependent induction of discoidin-I synthesis in exponentially growing cells of *Dictyostelium discoideum*. Differentiation **34:**79–87.
18. **Clarke, M., J. Yang, and S. Kayman.** 1988. Analysis of the prestarvation response in growing cells of *Dictyostelium discoideum.* Dev. Genet. **9:**315–326.
19. **Cooper, J. A.** 1994. Straight and narrow or tortuous and intersecting? Curr. Biol. **4:**1118–1121.
20. **Cubitt, A. B., S. Dharmawardhane, and R. A. Firtel.** 1993. Developmentally regulated changes in 1,2-diacylglycerol in *Dictyostelium*—regulation by light and G-proteins. J. Biol. Chem. **268:**17431–17439.
21. **Datta, S., and R. A. Firtel.** 1987. Identification of the sequences controlling cyclic AMP regulation and cell-type-specific expression of a prestalk-specific gene in *Dictyostelium discoideum*. Mol. Cell. Biol. **7:**149–159.
22. **Datta, S., and R. A. Firtel.** 1988. An 80-bp *cis*-acting regulatory region controls cAMP and developmental regulation of a prestalk gene in *Dictyostelium*. Genes Dev. **2:**294–304.

23. **Datta, S., R. H. Gomer, and R. A. Firtel.** 1986. Spatial and temporal regulation of a foreign gene by a prestalk-specific promotor in transformed *Dictyostelium discoideum*. Mol. Cell. Biol. **6:**811–820.
24. **Datta, S., S. K. O. Mann, A. Hjorth, R. Gomer, P. Howard, D. Armstrong, C. Reymond, C. Silan, and R. A. Firtel.** 1987. cAMP-regulated gene expression during *Dictyostelium* development is mediated by the cell-surface cAMP receptor, p. 33–61. *In* W. F. Loomis (ed.), Genetic regulation of development. Academic Press, Inc., New York.
25. **Davies, L., M. Satre, J. B. Martin, and J. D. Gross.** 1993. The target of ammonia action in *Dictyostelium*. Cell **75:**321–327.
26. **Davis, R.** 1993. The mitogen-activated protein kinase signal transduction pathway. J. Biol. Chem. **268:**14553–14556.
27. **de Groot, R., J. Auwerx, M. Bourouis, and P. Sassone-Corsi.** 1993. Negative regulation of Jun/AP1: conserved function of glycogen synthase kinase-3 and *Drosophila* kinase shaggy. Oncogene **8:**841–847.
28. **Devreotes, P. N.** 1994. G protein-linked signaling pathways control the developmental program of *Dictyostelium*. Neuron **12:**235–241.
29. **Dharmawardhane, S., A. Cubitt, and R. Firtel.** 1995. Regulatory role of G-alpha-1 subunit in controlling cellular morphogenesis in *Dictyostelium*. Development **120:**354–356.
30. **Diaz-Benjumea, F., and S. Cohen.** 1994. *wingless* acts through the *shaggy*/*zeste-white* 3 kinase to direct dorsal-central axis formation in the *Drosophila* leg. Development **120:**1661–1670.
31. **Dominguez, I., K. Itoh, and S. Y. Sokol.** 1995. Role of glycogen synthase kinase 3β as a negative regulator of dorsoventral axis formation in *Xenopus* embryos. Proc. Natl. Acad. Sci. USA **92:**8498–8502.
32. **Drayer, A. L., J. Vanderkaay, G. W. Mayr, and P. J. M. Vanhaastert.** 1994. Role of phospholipase C in *Dictyostelium*—formation of inositol 1,4,5-trisphosphate and normal development in cells lacking phospholipase C activity. EMBO J. **13:**1601–1609.
33. **Dynes, J. L., A. M. Clark, G. Shaulsky, A. Kuspa, W. F. Loomis, and R. A. Firtel.** 1994. LagC is required for cell-cell interactions that are essential for cell-type differentiation in *Dictyostelium*. Genes Dev. **8:**948–958.
34. **Early, A. E., M. J. Gaskell, D. Traynor, and J. G. Williams.** 1993. Two distinct populations of prestalk cells within the tip of the migratory *Dictyostelium* slug with differing fates at culmination. Development **118:**353–362.
35. **Early, A. E., and J. G. Williams.** 1989. Identification of sequences regulating the transcription of a *Dictyostelium* gene selectively expressed in prespore cells. Nucleic Acids Res. **17:**6473–6484.
36. **Early, A. E., J. G. Williams, H. E. Meyer, S. B. Por, E. Smith, K. L. Williams, and A. A. Gooley.** 1988. Structural characterization of *Dictyostelium discoideum* prespore-specific gene D19 and of its product, cell surface glycoprotein PsA. Mol. Cell. Biol. **8:**3458–3466.
37. **Early, V. E., and J. G. Williams.** 1988. A *Dictyostelium* prespore-specific gene is transcriptionally repressed by DIF *in vitro*. Development **103:**519–524.
38. **Errede, B., and D. Levin.** 1993. A conserved kinase cascade for MAP kinase activation in yeast. Curr. Opin. Cell Biol. **5:**254–260.
39. **Escalante, R., and W. F. Loomis.** 1995. Whole-mount *in situ* hybridization of cell-type specific mRNAs in *Dictyostelium*. Dev. Biol. **171:**262–266.
40. **Esch, R. K., P. K. Howard, and R. A. Firtel.** 1992. Regulation of the *Dictyostelium* cAMP-induced, prestalk-specific *DdrasD* gene—identification of *cis*-acting elements. Nucleic Acids Res. **20:**1325–1332.
41. **Faix, J., G. Gerisch, and A. A. Noegel.** 1992. Overexpression of the csA cell adhesion molecule under its own cAMP-regulated promoter impairs morphogenesis in *Dictyostelium*. J. Cell Sci. **102:**203–214.
42. **Faure, M., J. Franke, A. L. Hall, G. J. Podgorski, and R. H. Kessin.** 1990. The cyclic nucleotide phosphodiesterase gene of *Dictyostelium discoideum* contains three promoters specific for growth, aggregation, and late development. Mol. Cell. Biol. **10:**1921–1930.
43. **Firtel, R. A.** 1991. Signal transduction pathways controlling multicellular development in *Dictyostelium*. Trends Genet. **7:**381–388.
44. **Firtel, R. A.** 1995. Integration of signaling information in controlling cell-fate decisions in *Dictyostelium*. Genes Dev. **9:**1427–1444.

44a. **Firtel, R. A.** Personal communication.

45. **Fosnaugh, K. L., and W. F. Loomis.** 1989. Sequence of the *Dictyostelium discoideum* spore coat gene SP96. Nucleic Acids Res. **17:**9489–9490.
46. **Fosnaugh, K. L., and W. F. Loomis.** 1989. Spore coat genes SP60 and SP70 of *Dictyostelium discoideum*. Mol. Cell. Biol. **9:**5215–5218.
47. **Fosnaugh, K. L., and W. F. Loomis.** 1991. Coordinate regulation of the spore coat genes in *Dictyostelium discoideum*. Dev. Genet. **12:**123–132.
48. **Fosnaugh, K. L., and W. F. Loomis.** 1993. Enhancer regions responsible for temporal and cell-type-specific expression of a spore coat gene in *Dictyostelium*. Dev. Biol. **157:**38–48.
49. **Franke, J., M. Faure, L. Wu, A. L. Hall, G. J. Podgorski, and R. H. Kessin.** 1991. Cyclic nucleotide phosphodiesterase of *Dictyostelium discoideum* and its glycoprotein inhibitor—structure and expression of their genes. Dev. Genet. **12:**104–112.

49a. **Fuller, D., and W. F. Loomis.** Unpublished observations.

50. **Garrod, D. R., and J. M. Ashworth.** 1972. Effect of growth conditions on development of the cellular slime mould *Dictyostelium discoideum*. J. Embryol. Exp. Morphol. **28:**463–479.
51. **Gaskins, C., A. M. Clark, L. Aubry, J. E. Segall, and R. A. Firtel.** 1996. The *Dictyostelium* MAP kinase ERK2 regulates multiple, independent developmental pathways. Genes Dev. **10:**118–128.
52. **Gaskins, C., M. Maeda, and R. Firtel.** 1994. Identification and functional analysis of a developmentally regulated extracellular signal-regulated kinase gene in *Dictyostelium discoideum*. Mol. Cell. Biol. **14:**6996–7012.
53. **Gee, K., F. Russell, and J. D. Gross.** 1994. Ammonia hypersensitivity of slugger mutants of *Dictyostelium discoideum*. J. Cell Sci. **107:**701–708.
54. **Gilman, A. G.** 1987. G protein: transducers of receptor-generated signals. Annu. Rev. Biochem. **56:**615–649.
55. **Gomer, R., S. Datta, M. Mehdy, T. Crowley, A. Sivertson, W. Nellen, C. Reymond, S. Mann, and R. A. Firtel.** 1985. Regulation of cell-type specific gene expression in *Dictyostelium*. Cold Spring Harbor Symp. Quant. Biol. **50:**801–812.
56. **Gomer, R. H., and R. A. Firtel.** 1987. Cell-autonomous determination of cell-type choice in *Dictyostelium* development by cell-cycle phase. Science **237:**758–762.
57. **Gomer, R. H., I. S. Yuen, and R. A. Firtel.** 1991. A secreted 80×10^3 Mr protein mediates sensing of cell density and the onset of development in *Dictyostelium*. Development **112:**269–278.
58. **Gross, J. D., J. Bradbury, R. R. Kay, and M. J. Peacey.** 1983. Intracellular pH and the control of cell differentiation in *Dictyostelium discoideum*. Nature (London) **303:**244–245.
59. **Haberstroh, L., J. Galindo, and R. A. Firtel.** 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.
60. **Harwood, A., S. Plyte, J. Woodgett, H. Strutt, and R. Kay.** 1995. Glycogen synthetase kinase 3 (GSK-3) regulates cell fate in *Dictyostelium*. Cell **80:**139–148.
61. **Harwood, A. J., A. Early, and J. G. Williams.** 1993. A repressor controls the timing and spatial localisation of stalk cell-specific gene expression in *Dictyostelium*. Development **118:**1041–1048.
62. **Harwood, A. J., N. A. Hopper, M. N. Simon, S. Bouzid, M. Veron, and J. G. Williams.** 1992. Multiple roles for camp-dependent protein kinase during *Dictyostelium* development. Dev. Biol. **149:**90–99.
63. **Harwood, A. J., N. A. Hopper, M. N. Simon, D. M. Driscoll, M. Veron, and J. G. Williams.** 1992. Culmination in *Dictyostelium* is regulated by the cAMP-dependent protein kinase. Cell **69:**615–624.
64. **He, X., J. Saint-Jeanet, J. R. Woodgett, H. E. Varmus, and I. B. Dawid.** 1995. Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. Nature (London) **374:**617–622.
65. **Herskowitz, I.** 1995. MAP kinase pathways in yeast: for mating and more. Cell **80:**187–197.
66. **Hjorth, A. L., N. C. Khanna, and R. A. Firtel.** 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.
67. **Hjorth, A. L., C. Pears, J. G. Williams, and R. A. Firtel.** 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.
68. **Hopper, N. A., G. Sanders, K. Fosnaugh, J. Williams, and W. F. Loomis.** 1995. Protein kinase A is a positive regulator of spore coat gene transcription in *Dictyostelium*. Differentiation **58:**183–188.
69. **Hopper, N. A., and J. Williams.** 1994. A role for cAMP-dependent protein kinase in determining the stability of prespore cell differentiation in *Dictyostelium*. Dev. Biol. **163:**285–287.

69a. **Hopper, N. A., and J. Williams.** Personal communication.

70. **Howard, P. K., M. Gamper, T. Hunter, and R. A. Firtel.** 1994. Regulation by protein-tyrosine phosphatase PTP2 is distinct from that by PTP1 during *Dictyostelium* growth and development. Mol. Cell. Biol. **14:**5154–5164.
71. **Howard, P. K., B. M. Sefton, and R. A. Firtel.** 1992. Analysis of a spatially regulated phosphotyrosine phosphatase identifies tyrosine phosphorylation as a key regulatory pathway in *Dictyostelium*. Cell **71:**637–647.
72. **Insall, R., A. Kuspa, P. J. Lilly, G. Shaulsky, L. R. Levin, W. F. Loomis, and P. Devreotes.** 1994. CRAC, a cytosolic protein containing a pleckstrin homology domain, is required for receptor and G protein-mediated activation of adenylyl cyclase in *Dictyostelium*. J. Cell Biol. **126:**1537–1545.
73. **Insall, R., O. Nayler, and R. R. Kay.** 1992. DIF-1 induces its own breakdown in *Dictyostelium*. EMBO J. **11:**2849–2854.
74. **Insall, R. H., R. D. M. Soede, P. Schaap, and P. N. Devreotes.** 1994. Two cAMP receptors activate common signaling pathways in *Dictyostelium*. Mol. Biol. Cell **5:**703–711.
75. **Jain, R., and R. H. Gomer.** 1994. A developmentally regulated cell surface receptor for a density-sensing factor in *Dictyostelium*. J. Biol. Chem. **269:**9128–9136.
76. **Jermyn, K. A., K. T. Duffy, and J. G. Williams.** 1989. A new anatomy of the prestalk zone in *Dictyostelium*. Nature (London) **340:**144–146.
77. **Jermyn, K. A., and J. G. Williams.** 1991. An analysis of culmination in *Dictyostelium* using prestalk and stalk-specific cell autonomous markers. Development **111:**779–787.
78. **Johnson, R. L., R. Gunderson, D. Hereld, G. S. Pitt, S. Tugendreich, C. L. Saxe, A. R. Kimmel, and P. N. Devreotes.** 1992. G-protein linked signaling

pathways mediate development in *Dictyostelium*. Cold Spring Harbor Symp. Quant. Biol. **57:**169–176.
79. **Johnson, R. L., C. L. Saxe, R. Gollop, A. R. Kimmel, and P. N. Devreotes.** 1993. Identification and targeted gene disruption of CAR3, a cAMP receptor subtype expressed during multicellular stages of *Dictyostelium* development. Genes Dev. **7:**273–282.
80. **Kamboj, R. K., T. Y. Lam, and C. H. Siu.** 1990. Regulation of slug size by the cell adhesion molecule gp80 in *Dictyostelium discoideum*. Cell Regul. **1:** 715–729.
81. **Kay, R. R., S. Large, D. Traynor, and O. Nayler.** 1993. A localized differentiation-inducing-factor sink in the front of the *Dictyostelium* slug. Proc. Natl. Acad. Sci. USA **90:**487–491.
82. **Ken, R., and C. K. Singleton.** 1994. Redundant regulatory elements account for the developmental control of a ribosomal protein gene of *Dictyostelium discoideum*. Differentiation **55:**97–103.
82a. **Kessin, R.** Personal communication.
83. **Kieber, J., M. Rothenberg, G. Roman, K. Feldmann, and J. Ecker.** 1993. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. Cell **72:**427–441.
84. **Kimmel, A. R., and R. A. Firtel.** 1991. cAMP signal transduction pathways regulating development of *Dictyostelium discoideum*. Curr. Opin. Genet. Dev. **1:**383–390.
85. **Knecht, D. A., D. L. Fuller, and W. F. Loomis.** 1987. Surface glycoprotein, gp24, involved in early adhesion of *Dictyostelium discoideum*. Dev. Biol. **121:**277–283.
86. **Kumagai, A., J. A. Hadwiger, M. Pupillo, and R. A. Firtel.** 1991. Molecular genetic analysis of two Gα protein subunits in *Dictyostelium*. J. Biol. Chem. **266:**1220–1228.
87. **Kumagai, A., M. Pupillo, R. Gundersen, R. Miake-Lye, P. N. Devreotes, and R. A. Firtel.** 1989. Regulation and function of Gα protein subunits in *Dictyostelium*. Cell **57:**265–275.
88. **Kuspa, A., and W. F. Loomis.** 1992. Tagging developmental genes in *Dictyostelium* by restriction enzyme-mediated integration of plasmid DNA. Proc. Natl. Acad. Sci. USA **89:**8803–8807.
89. **Kuspa, A., and W. F. Loomis.** 1994. Transformation of *Dictyostelium*—gene disruptions, insertional mutagenesis, and promoter traps. Methods Mol. Genet. **3:**3–21.
90. **Kuwayama, H., S. Ishida, and P. Van Haastert.** 1993. Non-chemotactic *Dictyostelium discoideum* mutants with altered cGMP signal transduction. J. Cell Biol. **123:**1453–1462.
91. **Lilly, P., L. J. Wu, D. L. Welker, and P. N. Devreotes.** 1993. A G-protein beta-subunit is essential for *Dictyostelium* development. Genes Dev. **7:** 986–995.
92. **Lilly, P. J., and P. N. Devreotes.** 1994. Identification of CRAC, a cytosolic regulator required for guanine nucleotide stimulation of adenylyl cyclase in *Dictyostelium*. J. Biol. Chem. **269:**14123–14129.
93. **Loomis, W. F.** 1975. *Dictyostelium discoideum*: a developmental system. Academic Press, Inc., New York.
94. **Loomis, W. F.** 1987. Genetic tools for *Dictyostelium discoideum*. Methods Cell Biol. **28:**31–65.
95. **Loomis, W. F.** 1993. Lateral inhibition and pattern formation in *Dictyostelium*. Curr. Top. Dev. Biol. **28:**1–46.
96. **Loomis, W. F., C. Klein, and P. Brachet.** 1978. The effect of divalent cations on aggregation of *Dictyostelium discoideum*. Differentiation **12:**83–89.
97. **Louis, J., G. Ginsburg, and A. Kimmel.** 1994. A cAMP receptor regulates axial patterning and cellular differentiation during late development of *Dictyostelium*. Genes Dev. **8:**2052–2061.
98. **Louis, J. M., C. L. Saxe III, and A. R. Kimmel.** 1993. Two transmembrane signaling mechanisms control expression of the cAMP receptor gene CAR1 during *Dictyostelium* development. Proc. Natl. Acad. Sci. USA **90:**5969–5973.
99. **Maeda, M., L. Aubry, R. Insall, C. Gaskins, P. Devreotes, and R. Firtel.** 1996. Seven helix chemoattractant receptors stimulate MAP kinase in the absence of functional G-proteins in *Dictyostelium*. J. Biol. Chem. **271:**3351–3355.
100. **Maeda, T., M. Takekawa, and H. Saito.** 1995. Activation of the yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. Science **269:**554–558.
101. **Maeda, Y.** 1993. Pattern formation in a cell-cycle dependent manner during the development of *Dictyostelium discoideum*. Dev. Growth Differ. **35:**609–616.
102. **Mann, S. K. O., and R. A. Firtel.** 1991. A developmentally regulated, putative serine/threonine protein kinase is essential for development in *Dictyostelium*. Mech. Dev. **35:**89–101.
103. **Mann, S. K. O., and R. A. Firtel.** 1993. cAMP-dependent protein kinase differentially regulates prestalk and prespore differentiation during *Dictyostelium* development. Development **119:**135–146.
104. **Mann, S. K. O., D. L. Richardson, S. Lee, A. R. Kimmel, and R. A. Firtel.** 1994. 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.
105. **Mann, S. K. O., W. M. Yonemoto, S. S. Taylor, and R. A. Firtel.** 1992. DdPK3, which plays essential roles during *Dictyostelium* development, encodes the catalytic subunit of cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA **89:**10701–10705.
106. **McDonald, S. A.** 1986. Cell-cycle regulation of center initiation in *Dictyostelium discoideum*. Dev. Biol. **117:**546–549.
107. **McRobbie, S. J., K. A. Jermyn, K. Duffy, K. Blight, and J. G. Williams.** 1988. Two DIF-inducible, prestalk-specific mRNAs of *Dictyostelium* encode extracellular matrix protein of the slug. Development **104:**275–284.
108. **McRobbie, S. J., R. Tilly, K. Blight, A. Ceccarelli, and J. G. Williams.** 1988. Identification and localization of proteins encoded by two DIF-inducible genes of *Dictyostelium*. Dev. Biol. **125:**59–63.
109. **Mehdy, M. C., and R. A. Firtel.** 1985. A secreted factor and cyclic AMP jointly regulate cell-type-specific gene expression in *Dictyostelium discoideum*. Mol. Cell. Biol. **5:**705–713.
110. **Milne, J. L., and P. N. Devreotes.** 1993. The surface cyclic AMP receptors, cAR1, cAR2, and cAR3, promote Ca^{++} influx in *Dictyostelium discoideum* by a Gα2-independent mechanism. Mol. Biol. Cell **4:**283–292.
111. **Monod, J., and F. Jacob.** 1961. General conclusions: telonomic mechanisms in cellular metabolism, growth, and differentiation. Cold Spring Harbor Symp. Quant. Biol. **26:**389–401.
112. **Morris, H. R., G. W. Taylor, M. S. Masento, K. A. Jermyn, and R. R. Kay.** 1987. Chemical structure of the morphogen differentiation inducing factor from *Dictyostelium discoideum*. Nature (London) **328:**811–814.
113. **Morrison, A., R. Blanton, M. Grimson, M. Fuchs, K. Williams, and J. Williams.** 1995. Disruption of the gene encoding the EcmA, extracellular matrix protein of *Dictyostelium*, alters slug morphology. Dev. Biol. **163:** 457–466.
114. **Morrissey, J. H., K. M. Devine, and W. F. Loomis.** 1984. The timing of cell-type differentiation in *Dictyostelium discoideum*. Dev. Biol. **103:**414–424.
115. **Morrissey, J. H., P. A. Farnsworth, and W. F. Loomis.** 1981. Pattern formation in *Dictyostelium discoideum*: an analysis of mutants altered in cell proportioning. Dev. Biol. **83:**1–8.
116. **Morrissey, J. H., and W. F. Loomis.** 1981. Parasexual genetic analysis of cell proportioning mutants of *Dictyostelium discoideum*. Genetics **99:**183–196.
117. **Murray, B. A., S. Wheeler, T. Jongens, and W. F. Loomis.** 1984. Mutations affecting a surface glycoprotein, gp80, of *Dictyostelium discoideum*. Mol. Cell. Biol. **4:**514–519.
118. **Murray, B. A., L. D. M. Yee, and W. F. Loomis.** 1981. Immunological analysis of a glycoprotein (contact sites A) involved in intercellular adhesion of *Dictyostelium discoideum*. J. Supramol. Struct. Cell. Biochem. **17:** 197–211.
119. **Mutzel, R., M. Simon, M. Lacombe, and M. Veron.** 1988. Expression and properties of the regulatory subunit of *Dictyostelium* cAMP-dependent protein kinase encoded by lambda gt11 cDNA clones. Biochemistry **27:**481–486.
119a. **North, M., and J. G. Williams.** Personal communication.
120. **Ochiai, H., J. Stadler, M. Westphal, G. Wagle, R. Merkl, and G. Gerisch.** 1982. Monoclonal antibodies against contact sites A of *Dictyostelium discoideum*: detection of modifications of the glycoprotein in tunicamycin-treated cells. EMBO J. **1:**1011–1016.
121. **Okaichi, K., A. B. Cubitt, G. S. Pitt, and R. A. Firtel.** 1992. Amino acid substitutions in the *Dictyostelium* Gα subunit Gα2 produce dominant negative phenotypes and inhibit the activation of adenylyl cyclase, guanylyl cyclase, and phospholipase C. Mol. Biol. Cell **3:**735–747.
122. **Oyama, M., K. Kubota, and K. Okamoto.** 1991. Regulation of guanylate cyclase by a guanine nucleotide binding protein, Gα2, in *Dictyostelium discoideum*. Biochem. Biophys. Res. Commun. **176:**1245–1249.
123. **Ozaki, T., H. Nakao, H. Orii, T. Morio, I. Takeuchi, and M. Tasaka.** 1993. Developmental regulation of transcription of a novel prespore-specific gene (Dp87) in *Dictyostelium discoideum*. Development **117:**1299–1308.
124. **Pavlovic, J., B. Haribabu, and R. P. Dottin.** 1989. Identification of a signal transduction response sequence element necessary for induction of a *Dictyostelium discoideum* gene by extracellular cyclic AMP. Mol. Cell. Biol. **9:** 4660–4669.
125. **Pears, C. J., H. M. Mahbubani, and J. G. Williams.** 1985. Characterization of two highly diverged but developmentally coregulated cysteine proteinases in *Dictyostelium discoideum*. Nucleic Acids Res. **13:**8853–8866.
126. **Pears, C. J., and J. G. Williams.** 1987. Identification of a DNA sequence element required for efficient expression of a developmentally regulated and cAMP-inducible gene of *Dictyostelium discoideum*. EMBO J. **6:**195–200.
127. **Pierce, S. B., and D. Kimelman.** 1995. Regulation of Spemann organizer formation by the intracellular kinase Xgsk-3. Development **121:**755–765.
128. **Pitt, G. S., N. Milona, J. Borleis, K. C. Lin, R. R. Reed, and P. N. Devreotes.** 1992. Structurally distinct and stage-specific adenylyl cyclase genes play different roles in *Dictyostelium* development. Cell **69:**305–315.
129. **Podgorski, G. J., M. Faure, J. Franke, and R. H. Kessin.** 1988. The cyclic nucleotide phosphodiesterase of *Dictyostelium discoideum*: the structure of the gene and its regulation and role in development. Dev. Genet. **9:**267–278.
130. **Powell-Coffman, J., and R. Firtel.** 1994. Characterization of a novel *Dic-*

tyostelium discoideum prespore-specific gene, pspB, reveals conserved regulatory sequences. Development **120:**1601–1611.
131. **Powell-Coffman, J. A., G. R. Schnitzler, and R. A. Firtel.** 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.
132. **Raper, K. B.** 1940. Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. J. Elisha Mitchell Sci. Soc. **56:**241–282.
133. **Rathi, A., S. C. Kayman, and M. Clarke.** 1991. Induction of gene expression in *Dictyostelium* by prestarvation factor, a factor secreted by growing cells. Dev. Genet. **12:**82–87.
134. **Reymond, C. D., R. H. Gomer, M. C. Mehdy, and R. A. Firtel.** 1984. Developmental regulation of a *Dictyostelium* gene encoding a protein homologous to mammalian *ras* protein. Cell **39:**141–148.
135. **Reymond, C. D., R. H. Gomer, W. Nellen, A. Theibert, P. Devreotes, and R. Firtel.** 1986. Phenotypic changes induced by a mutated *ras* gene during the development of *Dictyostelium* transformants. Nature (London) **323:**340–343.
136. **Richardson, D. L., and W. F. Loomis.** 1992. Disruption of the sporulation-specific gene spiA in *Dictyostelium discoideum* leads to spore instability. Genes Dev. **6:**1058–1070.
137. **Richardson, D. L., and W. F. Loomis, and A. R. Kimmel.** 1994. Progression of an inductive signal activates sporulation in *Dictyostelium discoideum*. Development **120:**2891–2900.
138. **Saxe, C. L., G. T. Ginsburg, J. M. Louis, R. Johnson, P. N. Devreotes, and A. R. Kimmel.** 1993. CAR2, a prestalk cAMP receptor required for normal tip formation and late development of *Dictyostelium discoideum*. Genes Dev. **7:**262–272.
139. **Saxe, C. L., R. Johnson, P. N. Devreotes, and A. R. Kimmel.** 1991. Multiple genes for cell surface cAMP receptors in *Dictyostelium discoideum*. Dev. Genet. **12:**6–13.
140. **Saxe, C. L., Y. Yu, C. Jones, A. Bauman, and C. Haynes.** The cAMP receptor subtype cAR2 is restricted to a subset of prestalk cells during *Dictyostelium* development and is not induced by DIF-1. Dev. Biol., in press.
141. **Schnitzler, G., W. Fischer, and R. Firtel.** 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.
142. **Schnitzler, G. R., C. Briscoe, J. Brown, and R. A. Firtel.** 1995. Serpentine cAMP receptors may act through a G protein-independent pathway to induce postaggregative development in *Dictyostelium*. Cell **81:**737–745.
143. **Segall, J., A. Kuspa, G. Shaulsky, M. Ecke, M. Maeda, C. Gaskins, R. Firtel, and W. Loomis.** 1995. A MAP kinase necessary for receptor-mediated activation of adenylyl cyclase in *Dictyostelium*. J. Cell Biol. **128:**405–413.
143a. **Shaulsky, G., A. Kuspa, and W. F. Loomis.** 1995. A multidrug resistance transporter serine protease gene is required for prestalk specialization in *Dictyostelium*. Genes Dev. **9:**1111–1122.
144. **Shaulsky, G., and W. F. Loomis.** 1993. Cell type regulation in response to expression of ricin-A in *Dictyostelium*. Dev. Biol. **160:**85–98.
145. **Shaulsky, G., and W. F. Loomis.** Initial cell type divergence in *Dictyostelium* is independent of DIF-1. Dev. Biol., in press.
146. **Shaulsky, G., and W. F. Loomis.** 1995. Mitochondrial DNA replication but no nuclear DNA replication during development of *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. USA **92:**5660–5663.
146a. **Shaulsky, G., and W. F. Loomis.** Unpublished data.
147. **Singleton, C. K., C. E. McPherson, and S. S. Manning.** 1988. Deactivation of gene expression upon the onset of development in *Dictyostelium discoideum*. Dev. Genet. **9:**327–336.
148. **Snaar-Jagalska, B. E., F. Kesbeke, and P. J. M. Van Haastert.** 1988. G-proteins in the signal-transduction pathways of *Dictyostelium discoideum*. Dev. Genet. **9:**215–226.
149. **Soede, R. D. M., R. H. Insall, P. N. Devreotes, and P. Schaap.** 1994. Extracellular cAMP can restore development in *Dictyostelium* cells lacking one, but not two subtypes of early cAMP receptors (cARs). Evidence for involvement of cAR1 in aggregative gene expression. Development **120:**1997–2002.
149a. **Stege, J., and W. F. Loomis.** Unpublished data.
150. **Sun, T. J., and P. N. Devreotes.** 1991. Gene targeting of the aggregation stage cAMP receptor cAR1 in *Dictyostelium*. Genes Dev. **5:**572–582.
151. **Sussman, M.** 1987. Cultivation and synchronous morphogenesis of *Dictyostelium* under controlled experimental conditions. Methods Cell Biol. **28:**9–29.
152. **Takeuchi, I.** 1960. The correlation of cellular changes with succinic dehydrogenase and cytochrome oxidase activities in the development of the cellular slime molds. Dev. Biol. **2:**343–366.
153. **Valkema, R., and P. J. M. Van Haastert.** 1992. Inhibition of receptor-stimulated guanylyl cyclase by intracellular calcium ions in *Dictyostelium* cells. Biochem. Biophys. Res. Commun. **186:**263–268.
154. **Vaughan, R. A., and P. N. Devreotes.** 1988. Ligand-induced phosphorylation of the cAMP receptor from *Dictyostelium discoideum*. J. Biol. Chem. **263:**14538–14543.
155. **Vaughan, R. A., and C. L. Rutherford.** 1987. Distribution of cAMP-dependent protein kinase during development in *Dictyostelium discoideum*. Dev. Biol. **121:**359–367.
156. **Wang, N., J. H. Roelfsema, J. G. Williams, and P. Schaap.** 1990. Cytoplasmic acidification facilitates but does not mediate DIF-induced prestalk gene expression in *Dictyostelium discoideum*. Dev. Biol. **140:**182–188.
156a. **Wang, N., G. Shaulsky, and W. F. Loomis.** Unpublished data.
157. **Weijer, C. J., G. Duschl, and C. N. David.** 1984. Dependence of cell-type proportioning and sorting on cell cycle phase in *Dictyostelium discoideum*. J. Cell Sci. **70:**133–145.
157a. **Williams, J.** Personal communication.
158. **Williams, J. G., A. Ceccarelli, S. McRobbie, H. Mahbubani, R. R. Kay, A. Farly, M. Berks, and K. A. Jermyn.** 1987. Direct induction of *Dictyostelium* prestalk gene expression by D1F provides evidence that D1F is a morphogen. Cell **49:**185–192.
159. **Williams, J. G., K. T. Duffy, D. P. Lane, S. J. McRobbie, A. J. Harwood, D. Traynor, R. R. Kay, and K. A. Jermyn.** 1989. Origins of the prestalk-prespore pattern in *Dictyostelium* development. Cell **59:**1157–1163.
160. **Wu, L.-J., H. Kuwayama, P. J. M. van Haastert, and P. N. Devreotes.** 1995. The G protein beta-subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. J. Cell Biol. **129:**1667–1675.
161. **Yoder, B. K., and D. D. Blumberg.** 1994. The promoter of a gene encoding a novel *Dictyostelium* spore coat protein. Dev. Biol. **163:**38–48.
162. **Yoder, B. K., J. Mao, G. W. Erdos, C. M. West, and D. D. Blumberg.** 1994. Identification of a new spore coat protein gene in the cellular slime mold *Dictyostelium discoideum*. Dev. Biol. **163:**49–65.
163. **Yuen, I. S., C. Taphouse, K. A. Halfant, and R. H. Gomer.** 1991. Regulation and processing of a secreted protein that mediates sensing of cell density in *Dictyostelium*. Development **113:**1375–1385.