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 variants 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 $10^7$ 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
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, 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 prestavation 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, 136). 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 (pds4) 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 (pdi4) 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, A dispensable, 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. When the signal is removed from the environment by the activity of phosphodiesterase and the cells 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

### Table 1. Aggregation stage genes

| Gene    | Product (gene) | Function | Phenotype of null mutant | Reference |
|---------|----------------|----------|--------------------------|-----------|
| 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       |
| dagaA   | CRAC           | Signal relay | Lack of aggregation      | 72        |
| erkA    | MAP kinase     | Signal relay | Lack of aggregation      | 52        |
| erkB    | MAP kinase     | Signal relay | Lack of aggregation      | 143       |
| gpaB    | Go2            | 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        |
remain permanently desensitized and hence no longer responsible for activation of adenylyl cyclase. At this time, another cAMP receptor, CAR3, is expressed and coupled to trimeric G proteins containing Go2 (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 Go2, as shown by the lack of activation of guanylyl cyclase or accumulation of cGMP in strains carrying null mutations in the gene encoding Go2, gsaB (86, 121, 122). Likewise, overexpression of gsaB 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 Go 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 amoeboid 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 acA 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.
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
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 G2 phase, when they have grown almost enough to divide again. In such cell cycle-unsynchronized populations, cells in early G1 will preferentially become prestalk cells whereas cells in late G2 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 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

| 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    | Auxillary 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        |

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**TABLE 2. Postaggregation and cell type specialization stage genes**
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.
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 auto-regulated. 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 this 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, which in 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, GFB 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 enc-
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 segment 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-componentsystem 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

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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 | Few spores | 60 |
| gskA | GSK3    | Protein kinase | Impaired spores | 137 |
| spaA | SpiA    | Spore coat protein | Very long stalks; no spores | 115 |

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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 descend 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 spaA 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, 

$\text{dhkA}$ plays roles in both prestalk and prespore cells during culmination. Mutants in which this gene is inactivated express $\text{ecmO}$ 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, $\text{dhkA}$ appears to repress $\text{ecmO}$ 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 $\text{pkaA}$ mutant cells that are dissociated from spores 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, $\text{spiA}$, is induced and encapsulation ensues (137). Normally, $\text{spiA}$ 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 $\text{spiA}$ 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 $\text{tagB}$ 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 $\text{dhkA}$ 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 $\text{stalky (stkA)}$ (116). It is likely that there is only a single locus which can mutate to give the $\text{stalky}$ phenotype. The gene has been disrupted by plasmid insertion, cloned, and sequenced (14a). $\text{stkA}$ 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 $\text{stkA}$ strains not encapsulate, but they also fail to express the sporulation marker gene $\text{spiA}$ 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, $\text{ecmA}$ and $\text{ecmB}$, $\text{ST430}$ and $\text{ST310}$, 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 $\text{ecmA}$ 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 $\text{dhkA}$ 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 $\text{dhkA}$ plays a role in PST-A cells as well as in prespore cells. In wild-type PST-A cells, it acts on the $\text{ecmO}$ cis-acting sites to repress transcription during culmination. In mutants in which $\text{dhkA}$ 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 $\text{ecmO::lacZ}$, cells at the tip are stained only at the end of culmination when the fruiting body is completed. $\text{ecmB}$ 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 $\text{ecmB}$ 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 $\text{ecmB::lacZ}$. Cells in the upper cup, which is formed from anterior-like cells during culmination, are also stained in strains carrying $\text{ecmB::lacZ}$. Expression in these cells is dependent on a 737-bp region that is found about 1 kb upstream of the transcriptional start site of $\text{ecmB}$ (12). A 278-bp region proximal to the transcriptional start site of $\text{ecmB}$ 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 $\text{pkaA}$ both drop precipitously when cells enter the top of the stalk tube and repression of $\text{ecmB}$ is relieved (104, 155).

Both $\text{ecmA}$ and $\text{ecmB}$ 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, $\text{ecmA}$ is expressed at a low level but $\text{ecmB}$ 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 $\text{cotB::lacZ}$ and $\text{ecmA::lacZ}$, which define prespore and prestalk cells, and yet DIF cannot be measured at that stage. Moreover, these reporter constructs are expressed in $\text{tagB}$ 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, $\text{carB}$ or $\text{tagB}$, 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 $\text{ecmA}$ and $\text{ecmB}$ (9). The rate of synthesis of $\text{ST430}$ and $\text{ST310}$, the products of the $\text{ecmA}$ and $\text{ecmB}$ 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 NH₄⁺, would have the opposite effect, and NH₄⁺ 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 Gtα1, since the increase in 1,2-diacylglycerol level is reduced in strains lacking Gtα1 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 _dhhA_ (156a). _dhhA_ 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 _dhhA_ 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, SNL1, inhibits the response regulator, SSK1 (19, 65). Under conditions of high osmolality, SNL1 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 _dhhA_ 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 _carB_ and, perhaps, also those encoded by _carD_ (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 (93, 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 may 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.
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 permitting 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 naïve 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 insensitivethat 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 β 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 suppressors 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.

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