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Control of spatial patterning and cell-type proportioning in *Dictyostelium*

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The spatial patterning of prestalk and prespore cells in the slug arises from the differential sorting of newly differentiated cell types as the mound forms. This pattern is highly organized along an anterior–posterior axis and is constant irrespective of the size of the organism. Cell-type differentiation is plastic until late in development. A change in the ratio of cell types resulting from removal of part of the slug leads to a rapid restoration of the original ratio of the cell types through a pathway involving dedifferentiation, redifferentiation, and sorting of the existing cells. This review provides insight into various molecules, morphogens, and pathways regulating spatial patterning and cell-type proportioning.

Key words: spatial patterning / cell-type proportioning / cell sorting / cell-type differentiation

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Introduction

MULTICELLULAR DEVELOPMENT in *Dictyostelium* is the result of the chemotactic aggregation of $\sim 10^5$ cells to form a multicellular organism. During aggregation, nanomolar oscillatory pulses of extracellular cAMP initiated from an aggregation center are relayed outward by the sequential activation of adenylyl cyclase and the release of cAMP. Cells respond by chemotaxing inward and relaying the extracellular signal outward through the monolayer of cells (see these reviews for details).[1–3] As the aggregate forms, there is a switch in the signaling pathways from those that mediate aggregation to those that control post-aggregative and cell-type-specific gene expression. Extracellular cAMP levels rise to micromolar levels, leading to the adaptation of the aggregation-stage pathways and activation of post-aggregative pathways. The center of this developmental switch is the activation of the transcription factor GBF, which is required for the expression of all known post-aggregative and cell-type-specific genes.[4,5] Contemporaneous with GFP activation is the tyrosine phosphorylation and nuclear localization of the STAT transcription factor Dd-STATa.[6] A tip emerges on the top of the mound, and elongates to a motile slug in which prestalk and prespore cells are organized along the anterior-posterior axis. Eventually, the slug culminates into a fruiting body in which a spore mass is upheld on an elongated stalk.

In the last few years, a major effort has gone into understanding the pathways required for the initial induction of the cell types as well as the genes that are required for the initial patterning and maintenance of the spatial organization within the multicellular organism. This analysis has been enabled by the ability to use genetic and molecular genetic approaches to identify new genes and investigate their functions in the initial cell-fate decisions, maintenance, and control of spatial patterning. This chapter will examine the pathways and our current understanding of their regulation.

Organization of the cell types within the multicellular organism

The spatial organization of the cell types within the slug results from the differential sorting of cell types after the mound is formed. PstA and pstO cells, prestalk cell types, sort to the apical region, the prespore cells are in the middle, and the pstB cells, another prestalk cell type, are found in the base of the tipped aggregate. Continued morphogenesis results in the elongation of this tipped aggregate to

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form a finger that falls over to produce a migrating slug or pseudoplasmodium.[7–10] The pattern within the slug is highly organized along an anterior–posterior axis and is fixed, irrespective of the size of the organism, in wild-type strains (Figure 1). The pstA and pstO cell types, which comprise ~20% of the anterior of the slug, are defined by their expression of domains of the promoter from the prestalk-specific gene *ecmA*. The entire promoter, ecmAO, is expressed in pstA and pstO cells, whereas the ecmA and ecmO subdomains are specifically expressed in pstA and pstO cells, respectively.[11] In addition, there is a small cone of cells embedded within the pstA domain, designated pstAB cells.[11–14] pstAB cells express both *ecmA / lacZ* and *ecmB/ lacZ*.

The posterior ~80% of the slug is the prespore domain and is composed mostly of prespore cells. Anterior-like cells (ALCs), a prestalk-like cell population which express prestalk markers and some anterior-like, cell-type-specific markers, are found scattered throughout the prespore domain and constitute ~10% of the total cells of the organism. At the very posterior of the organism are the 'rearguard' cells that are predominantly prestalk B (pstB) cells with an intermixing of ALCs.[15–17] In addition, a group of pstB cells are localized at the bottom of the slug where it touches the substratum near the intersection of the pstO and prespore domains, suggesting a crude[18] dorsoventral organization within the slug. PstB cells specifically express the prestalk-specific gene *ecmB* (Figure 1).

Evidence for the regulation of a cell-type proportioning mechanism

The organization of cell types within the slug is highly defined and constant in slugs ranging in size from < 100 cells to ~10^5 cells. Thus, cell-type differentiation in *Dictyostelium* must be controlled through pathways that can properly regulate patterning in organisms with extreme differences in the number of participating cells (Figure 2). Evidence that cell-type patterning and proportioning in *Dictyostelium* is regulated through soluble morphogens and cell–cell interactions comes from the classic experiments performed by Raper a half a century ago.[19] In these experiments, Raper dissected the slug into an anterior prestalk component and a posterior prespore component. If either section is allowed to differentiate immediately, the anterior forms a fruiting body composed predominantly of stalk cells and the posterior forms a normally proportioned fruiting body. However, if the prestalk part is allowed to migrate, a somewhat normally proportioned fruiting body is formed. These results strongly suggest that the cell types are capable of sorting and dedifferentiating and redifferentiating to form a properly proportioned fruiting body in the absence of cell proliferation. Further use of a vital dye, neutral red, revealed the presence of ALCs (because they stain red, as do anterior prestalk cells, with vital dye), scattered in prespore domain (in the posterior fragment of the slug in Raper's experiment), which sorts out, moves to the anterior, and eventually forms a normally proportioned fruiting body. This explains the rapid regulation in the posterior fragment. This pathway has been termed 'transdifferentiation' and the relationships of the cell types in this pathway have been defined (pstA ⇔ pstO ⇔ ALC ⇔ prespore cells, see Figure 3).[10,20] How these ALCs are maintained in the posterior domain of the uncut slug is not clear.[10] Analyses strongly suggest that this regulation must be mediated, in part, by soluble morphogens. Differential chemotaxis towards cAMP by various cell types in the organism is one of the mechanisms which control cell-type position and patterning.

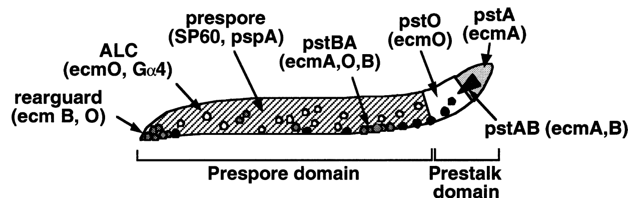

Figure 1. Distribution of cell types in the *Dictyostelium* slug. Localization of various cell types is defined using cell-type-specific antibodies and *lacZ* reporter constructs expressed from promoters from different cell-type-specific genes. See text for details.

Control of cell-type induction

Induction of prestalk and prespore cells is thought to result from a complex interaction of cAMP and the prestalk/stalk-inducing factor DIF, a chlorinated hexaphenone.[21–23] Once post-aggregative-stage genes are induced, cAMP induces prespore-specific gene expression. DIF is antagonistic toward prespore cell differentiation and is required for the induction of the pstB subclass of prestalk genes. DIF-mediated pstB cell differentiation is antagonized by extracellu-

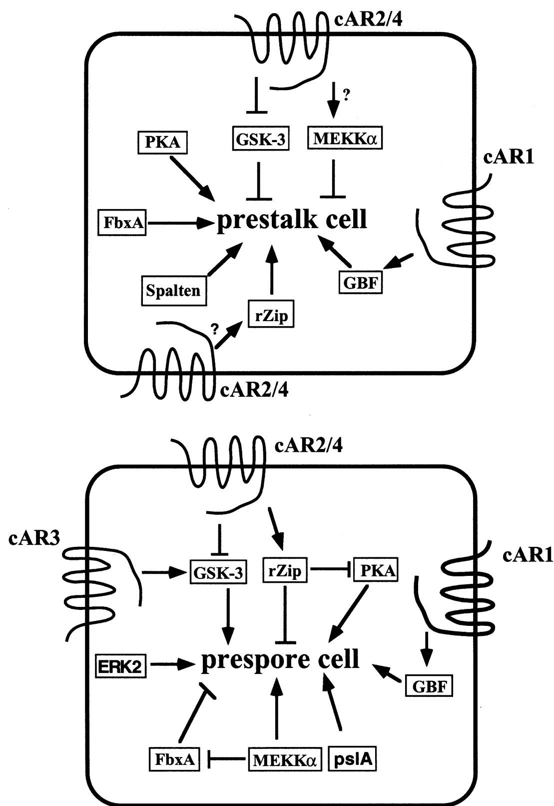

Figure 2. Regulation of cell-type differentiation and pattern formation. The cartoon illustrates the regulatory functions of genes controlling prestalk and prespore proportioning and cell-fate decisions. See text for details.

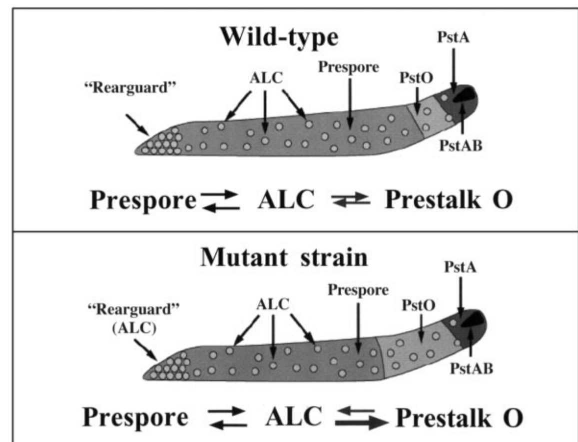

Figure 3. A cartoon describing the spatial patterning of strains lacking the homeobox containing gene Wariai or the F-box/WD40 repeat containing MEK kinase MEKKα as examples of patterning changes. The organization of cell types in the wild-type slug and in the mutant slug in which prespore and pstO compartment size is misregulated. The cartoon depicts the β-gal histochemical analysis of spatial patterning of prestalk and prespore cells in mutants such as *wariai* and *mekkα* null strains in which the prespore domain is reduced and the pstO compartment is expanded. Conversely, an increase in the prespore domain and a reduction in the pstO domain are found in *fbxA* and *rzip* null strains (data not shown).

lar cAMP.[21] PstA and pstO differentiation requires both DIF- and cAMP-mediated responses. The pathways controlling DIF- and cAMP-mediated responses in various cell types are discussed in more detail in other chapters.

Insights into pathways that regulate prestalk and prespore cell-fate decisions have resulted from genetic screens. Table 1 summarizes the functions of a number of identified genes known to be important in controlling cell-fate decisions and spatial patterning. The cell-type interactions regulated by these genes are shown in Figure 2. The gene *Spalten* (*Spn*) is required autonomously for prestalk cell differentiation and cell non-autonomously for prespore-specific gene expression.[24] Spalten is a modular protein containing an N-terminal domain that is homologous to Gα protein subunits of heterotrimeric G proteins, an inter-region domain, and a C-terminal PP2C serine/threonine phosphatase, which is the effector domain of the protein. *spn* null cells form aggregates that disperse but exhibit no induction of prestalk or prespore cell differentiation even in suspension culture in response to either cAMP or DIF, indicating that the inability to induce cell types is not a result of being unable to produce these morphogens. Significant understanding of the role of genes in controlling cell-fate decisions and patterning can be obtained through the use of chimeric organisms. In *Dictyostelium*, chimeras are produced by allowing cells of two strains to co-aggregate to produce a multicellular organism. By changing the input ratio of the two strains, one can vary the ratio of the cells from each strain in the resulting chimeric organisms. Such studies allow one to determine the developmental potential of strains to examine autonomous and non-autonomous defects in cell-type differentiation and patterning. Experiments using *spn* null cells in chimeras with wild-type cells demonstrate that *spn* null cells are unable to differentiate into prestalk cells but are able to form prespore and spore cells. This observation was highlighted in chimeric experiments with the null mutant of PslA, a nuclear factor that is required for prespore but not prestalk cell differentiation.[25] In chimeras with *pslA* null cells, *spn*

Table 1. Summary of cell-type-specific genes and factors required for prestalk and prespore patterning and differentiation (see text for references)

Genes	Homology	Effects of null on prespore cell/domain	Effects of null on prestalk cells/domain	Null phenotype	Comments
FbxA	Novel with an F-box and WD40 repeats	Increase in prespore domain (autonomous)	Decrease in pstO domain (autonomous)	Slugger (stays as a migrating slug), few fruiting bodies	May regulate targeted disruption of ubiquitinated protein
rZip	Possible adaptor protein: contains ring finger, SH3 domain, leucine zipper	Increase in prespore domain (non-autonomous and autonomous)	Decrease in ecmA and ecmB (autonomous)	Delayed development, larger aggregation stream with few centers	Regulates prestalk, prespore patterning, regulates PKA function
Wariai	Homeobox-containing transcription factor	Decrease in prespore domain	Increase in pstO domain (non-autonomous)	Enlarged basal disk and lower stalk region, ~ 30% arrest at slug stage	Regulates pstO and prespore cell ratios
Dd-STATa	STAT transcription factor	Decreased prespore domain in late slug	Increase in pstO domain, expanded expression of ecmB into the pstA region	No stalk cell differentiation or stalk tube formation *in vivo*, stalk cells induced *in vitro*	*stata* null cells are hypersensitive to cAMP for inhibition of stalk cell differentiation
Spalten	Contains a Gα subunit-like domain and a PP2C phosphatase domain	No prespore gene expression (non-autonomous)	No prestalk gene expression (autonomous)	Loose mound	Required for initial induction of prestalk cells and ALCs
MEKKα	MAP kinase kinase kinase, F-box and WD40 repeats	Decrease in prespore domain (non-autonomous)	Increase in pstO domain (autonomous)	Precocious development, smaller sori on longer stalk	Regulates pstO and prespore cell ratios, shows spatially and temporally restricted pattern of degradation
GSKA	GSK-3	Decrease in prespore gene expression (autonomous)	Increase in pstB expression	Large basal disk with no spore	Required for prespore and spore pathways
pslA	Nuclear factor, possible transcription factor	No prespore domain or prespore-specific gene expression (autonomous)	Reduced ecmA expression, increased ALC population	Fruiting body forms with no spores	May also be required for perception of signal in prestal cells for proper patterning
Gα4	Gα subunit coupled to folate/pterine receptors	Decrease in prespore gene expression (non-automonous)	Probable reduction of prestalk domain, *RasD/lacz* but not *ecmA/lacZ* not examined in original paper	Distorted upward extension of first finger	Regulates signaling downstream from folate/pterine receptors, distinct receptors function in early and later development

Table 1. (*Continued*)

cAR2	Cell-surface serpentine receptor for cAMP	Increase in prespore expression	Increase in ecmA, decrease in ecmB	Arrests at mound stage	Low affinity cAMP receptor, may regulate GSKA negatively
cAR3	Cell-surface serpentine receptor for cAMP	Decrease in pspA	Increase in ecmB	No significant morphological phenotype, patterning defects similar to those of GSKA null	Moderate affinity cAMP receptor, positively regulates GSKA
cAR4	Cell-surface serpentine receptor for cAMP	Increase in prespore domain, (non-autonomous)	Decrease in ecmA, ecmB (autonomous)	Mispatterned and diformed slug, mislocalization of prespore cells in prestalk region	Low affinity cAMP receptor, may regulate GSKA negatively
HP1	Not known	Decrease in prespore	Increase in ecmB	Not known	Gene not cloned

null cells form prespore cells and *pslA* null cells form prestalk cells and normally proportioned fruiting bodies can be obtained. These results indicate that Spalten is essential for production of the prestalk cell pathway but functions non-autonomously to regulate prespore cell differentiation. The results also indicate that effective prespore differentiation *in vivo* requires prestalk cell differentiation.[24]

Analysis of Spalten function indicates that the Gα-like domain directs Spalten to the plasma membrane and its GTP binding controls the function of the phosphatase effector domain without affecting Spalten targeting. This analysis suggests a model in which a protein, which is phosphorylated by an upstream kinase, is dephosphorylated by Spalten and then is required for entry into the prestalk cell differentiation pathway. This gene, ARCK1, encodes a serine/threonine kinase with structural homologies to metazoan Raf-1. These homologies include a putative CRD (cysteine-rich domain) that interacts with plasma membrane phospholipids and Ras proteins, a Raf-related kinase domain, a 14-3-3 interacting domain, and ankyrin repeats, the latter of which are not found in Raf-1. Overexpression of ARCK1 produces a Spalten-like phenotype, strongly suggesting that ARCK1 is the kinase that phosphorylates the Spalten substrate. Pathways that lie upstream of Spalten and ARCK1 are not presently known. An understanding of these should further elucidate mechanisms of the initial induction of prestalk and prespore cells.

Establishment of spatial patterning

A number of studies suggest that individual cells enter the multicellular aggregate with a propensity to differentiate into either prestalk or prespore cells. Experiments using synchronized cells suggest that cells in S or early G2 phase (*Dictyostelium* has no G1 phase in the cell cycle) preferentially differentiate into prestalk cells, whereas those in mid and later G2 differentiate into prespore cells.[26–30] This finding was extended to indicate that these effects are non-autonomous through the use of time-lapse video microscopy to examine the effect of the cell cycle in controlling the fate of isolated, separated cells or synchronized cells. Additional experiments using synchronized cells or cells grown under different conditions indicate that other factors, possibly in addition to the position in the cell cycle, have input on the propensity to differentiate into prestalk or prespore cells. Experiments in which either early or late populations are allowed to separately form a multicellular organism indicate that, even though cells from different stages of the cell cycle have a propensity to differentiate into one cell type or another, a slug containing only early or late cells forms almost normally proportioned fruiting bodies.[31–34] These results suggest that cell–cell interactions and morphogens within the multicellular aggregate modulate this initial patterning information to control proper proportioning.[27,28,35]

Molecular analysis suggests that this propensity to differentiate into prestalk or prespore cells correlates with the expression of genes involved in signaling pathways that mediate aggregation several hours later in development. 'Early' cells have higher concentrations of many of the components of the signaling pathways than late cells that are required for aggregation. This corresponds with the ability of earlier cells to preferentially initiate the formation of multicellular aggregates. Experiments suggest that expression of RasD, a gene expressed during growth and in prestalk cells during post-aggregative development, is differentially regulated in the cell cycle, being high in 'early' cells and low in 'late' cells.[36–38] These observations do not prove that higher levels of the expression of genes in S and early G2 phases are responsible for the propensity of these cells to differentiate into prestalk cells, nor does it indicate that the lower level of expression of these genes 'restricts' the differentiation of late cells to form prespore cells. However, the correlation between these expression levels and cell-type differentiation strongly suggests there is cell-cycle regulation of gene expression which, along with cAMP and DIF, regulate the information that is involved in controlling the initial proportioning of prestalk and prespore cells.

Regulation of cell fate and patterning by PKA and GSK3

cAMP-dependent protein kinase (PKA) regulates multiple pathways at key times in development, including aggregation, cell-type differentiation and patterning, and terminal differentiation.[39,40] Through genetic and biochemical approaches, PKA was found to be essential for prespore cell differentiation and required for maximal and efficient prestalk cell differentiation.[40–44] In suspension cultures, *pka* null cells are unable to induce prespore cell differentia-

tion in response to cAMP. Prestalk cell differentiation is induced at very low levels, but this can be increased through the overexpression of the transcription factor GBF.[45] Although activation of post-aggregative genes by GBF does not require PKA activity, GBF presumably functions in association with PKA-mediated pathways to control cell-type differentiation. Interestingly, there are two classes of prespore cells, one which requires PKA function and the other which does not; both sets of genes require the function of GBF to be induced and the nuclear factor PslA.[25] Analysis of *pslA* null cells suggests that after prespore cell differentiation is initiated, there is a divergence in the pathway with PKA-dependent and PKA-independent branches.

Two sets of early experiments indicate that the prespore domain is not 'homogeneous'.[46] The prespore-specific gene *SP60/CotC* has three GBF binding domains (G-box) that are required for efficient expression of the gene. Interestingly, deletion of the G-box in sequential order from 5′ to 3′ results in a gradient of *SP60* gene expression that is highest closer to the pstO region and lowest closer to the posterior of the slug. Sequential deletions starting at the 3′ end of the GBF binding region result in the opposite gradient. Transplantation experiments in which cells from the anterior part of the prespore domain or the posterior part of the prespore domain of one organism are transplanted into another organism show that these cells preferentially sort back to the anterior or posterior regions.

Recent analysis of rZIP, a gene encoding a putative adapter protein containing an SH3 binding domain, a leucine zipper, and a RING finger domain, suggests that differences in the prespore domain are the result of a gradient of PKA activity.[47,48] Disruption of rZIP levels leads to a reduction in prestalk-specific gene expression and an ~10-fold enhancement of prespore gene expression. This level of increase in prespore gene expression cannot be due to a conversion of prestalk to prespore cells (there are 75% fewer prestalk than prespore cells). Conversely, overexpression of rZIP leads to the opposite effect. Analysis of *rzip* null, rZIP overexpressor, and chimeric organisms carrying cell-type-specific promoter reporter constructs reveals that rZIP is involved in controlling prestalk/prespore patterning. Wild-type and *rzip* null strains exhibit a homogeneous expression of *SP60/CotC* in the posterior region of the slug. In chimeric organisms composed of 10% *rzip* null cells carrying the *SP60/lacZ* reporter and 90% wild-type cells, β-gal staining is restricted to the most anterior part of the prespore domain, even though the *rzip* null cells are uniformly distributed throughout the prespore region. This patterning is reminiscent of deletion analysis of the *SP60* promoter.[46] Addition of the membrane-permeable cAMP 8-Br-cAMP and an inducer of PKA activity to the rZIP/wild-type chimeric slugs induces expression in all *rzip* null cells in the prespore domain, indicating all *rzip* null cells are competent to respond to PKA activation. These results suggest the existence of a non-autonomous prespore activating signal responsible for maintaining prespore cell differentiation. Interestingly, only prespore-specific genes that require PKA for their transcriptional activation are subject to such regulation, suggesting PKA is a component of the extracellular pathway that regulates this process.[47,48]

Role of GSK3

As in metazoans, the serine/threonine kinase GSK3 is required for spatial patterning and cell-fate decisions in *Dictyostelium*.[49] *gsk3* null cells preferentially differentiate into the basal disk at the expense of prespore cells. Recent studies indicate that GSK3 lies downstream from cAMP receptor cAR3.[50] *car3* null cells exhibit patterning defects reminiscent of those of the *gsk3* null cells, except that the defects are not as severe,[51] possibly due to the basal activity of GSK3 and/or compensation by the cAMP receptor cAR1.

GSK3 activity increases during mound formation. The up-regulation is not observed in *car3* null cells and is induced in response to cAMP. The results suggest that cAR3 lies upstream from GSK3 and is required for its activation.[50] Recently, a novel kinase, ZAK1, which has a serine/threonine and a tyrosine kinase domain, has been implicated in the regulation of GSK3 activity (Kim L, Liu J, and Kimmel AR, sub. for pub.). ZAK1 functions downstream from the cAMP receptor cAR3 to activate GSK3 activity by phosphorylation of GSK3 on a tyrosine residue. Moreover, ZAK1 phosphorylates mammalian GSK3 on a tyrosine residue known to be required for GSK3 activation. In *zak1* null cells, GSK3 activity is not activated in response to cAMP and produces phenotypes similar to those of *car3* null cells: *zak1* nulls have reduced prespore gene expression and spore differentiation, are insensitive to cAMP repression of *ecmB* prestalk gene expression and stalk differentiation, and exhibit

spatial expansion of *ecmB* reporter gene expression. Furthermore, genetic studies indicate that the developmental functions of ZAK1 require wild-type GSK3.

In addition to being positively regulated by cAR3, GSK3 may be negatively regulated by cAR2 and cAR4.[52] cAR2 is specifically expressed in the prestalk cells. *car2* null cells arrest at the mound stage and exhibit aberrant cell-type-specific gene expression. Expression of *ecmA* and *ecmB* is reduced, whereas expression of prespore genes is ~10-fold higher than that in wild-type cells. Such large increases in prespore-specific gene expression cannot result from a differentiation of prestalk into prespore cells, but must result from a major increase in prespore-specific gene expression within prespore cells. *car4* null cells exhibit a similar pattern of gene expression.[53,54] Unlike *car2* null cells, *car4* null cells complete development, although there is a delay at culmination and the slug and fruiting bodies are quite abnormal. Analysis of spatial patterning shows that prespore cells are found in the anterior region of the slug that normally contains only prestalk cells. These deficits are not rescued by either DIF or cAMP. Analysis of chimeric organisms suggests that cAR4, which is preferentially but not exclusively expressed in prestalk cells, regulates prespore gene expression via a cell non-autonomous pathway. Conditioned medium from *car4* null cells has the ability to antagonize DIF repression of prespore-specific gene expression. Thus, cAR4 may function on the pathway that regulates the differential responsiveness of cells to DIF and/or cAMP. It is interesting to note that although *car4* and *rzip* null strains have a similar increase in prespore gene expression, it appears that in wild-type cells, cAR4 and rZIP function antagonistically in the non-continuous regulation of prespore cell differentiation.[50]

Linkage of the cAMP receptors to the GSK3 pathway has been obtained through experiments using LiCl.[52] LiCl is a potent inhibitor of GSK3 activity and addition of LiCl to wild-type cells mimics the *gsk3* null phenotype. When *car4* null cells are treated with an increase in the concentration of LiCl, prespore- and prestalk-specific gene expression is restored to wild-type levels. These results are consistent with cAR4 negatively regulating GSK3 activity.[49] GSK3 is thought to be important in the cAMP repression of stalk cell differentiation. *In vitro* cell culture experiments indicate the cAMP repression is more efficient in *car2* null cells than in wild-type cells. These results are consistent with cAR2 negatively regulating GSK3 activity, as does cAR4.

Regulation of pstO and prespore cell proportioning

Homeobox-containing genes regulate cell-type choice and cell patterning along the anterior/posterior axis in developing metazoans. Two homeobox-containing genes in *Dictyostelium*, *DdHbx-1* (*Wariai*) and *DdHbx-2*, have been identified.[55] Expression of both genes is induced at the mound stage by cAMP acting through receptor cAR1 in a GBF-dependent fashion. Mutational analysis of Wariai indicates it is essential in controlling the proportions of prestalk and prespore cells. In *wariai* null cells, there is an ~2.5-fold increase in the number of pstO cells and the size of the pstO domain and a concomitant decrease in prespore cells and the size of the prespore domain. *Ddhbx-2* null cells have no overt phenotype; however, a double knockout of *Wariai* and *DdHbx-2* has a more severe phenotype than *wariai* null cells alone, suggesting it functions on the same regulatory pathway. In chimeras, *wariai* null cells appear to induce an increase in the size of the domain exhibited by wild-type prestalk O cells, supporting a model in which *Wariai* non-autonomously controls pstO/prespore patterning (Figure 3).

Unexpectedly, *Wariai* is specifically expressed in pstA cells and there is no detectable expression in pstO or prespore cells. The *wariai* null phenotype can be complemented by expressing *Wariai* from the pstA-specific *ecmA* promoter but not from either the *ecmO* pstO-specific promoter or the prespore-specific promoter SP60/CotC. These experiments strongly suggest that *Wariai* non-autonomously controls cell-type proportioning.

Structurally, *Wariai* contains a homeobox gene and seven ankyrin repeats at the C-terminal tail of the protein. The function of both domains is required for *Wariai* function. It is expected, although not proven, that the ankyrin repeats act as a protein–protein interaction domain to form a complex with other proteins involved in mediating *Wariai* function. Although the mechanism of *Wariai* function is not known, it is expected that genes downstream from *Wariai* include those that could produce a morphogen involved in regulating pstO/prespore patterning.

Regulation of cell-type proportioning via signaling pathways and targeted protein degradation

MEKKα is the putative MEK kinase (MAP kinase

kinase kinase) which is a component of MAP kinase cascades. MEKKα regulates developmental timing and cell-type proportioning.[56] *mekk*α null cells develop rapidly and exhibit patterning defects that are similar to those of *wariai* null cells except that the *mekk*α null slugs lack a defined pstO/prespore boundary. It is possible that the differential rates of motility of the individual cell types, which are thought to play a role in patterning, or possible changes in cell adhesion are altered so prestalk and prespore cells in *mekk*α null strains are unable to effectively sort. In chimeras with wild-type cells, *mekk*α null cells initially induce prespore cells, as determined by the expression of SP60/GFP, but this expression is lost by the late slug stage. This loss of prespore gene expression is thought to result from a dedifferentiation of the prespore cells and the possible (although not demonstrated) differentiation of these cells into pstO cells. In reciprocal experiments using chimeras containing cells overexpressing MEKKα and wild-type cells, wild-type cells initially induce prespore gene expression, but the expression is lost as development proceeds. These analyses strongly suggest that MEKKα functions non-autonomously to produce and/or respond to a secreted factor (Figure 3).

MEKKα has a short, putative N-terminal regulatory domain, a kinase domain homologous to those found in MEK kinases, and a C-terminal domain that contains an F-box followed by WD40 repeats. F-boxes target proteins to degradation via the ubiquitination pathways in which F-boxes interact with Skip1, a component of the SCF complex.[57–59] The WD40 repeats are thought to be involved in protein–protein interactions and, in the case of MEKKα, these domains target the protein to the plasma membrane. Two-hybrid screens identified other components of the pathway. One is UBC1, a ubiquitin-conjugating enzyme that was previously identified as the protein required for the transition from the mound stage to multicellular differentiation and for culmination.[60] *ubc1* null cells have an increased number of pstB cells and exhibit aberrant cell-type patterning. The second component is UBP1, a deubiquitinating enzyme which removes ubiquitin from proteins and thus protects them from proteosome degradation.[56] Cell biological analyses demonstrated a direct role for UBC1 in targeting MEKKα for ubiquitination and degradation, whereas UBP1 reduces the level of MEKKα ubiquitination and stabilizes MEKKα.

Using GFP fusions, the MEKKα F-box/WD40 repeat is preferentially degraded in the prespore domain of migrating slugs and is stabilized in the anterior prestalk domain, although it is unclear if this stabilization is specific for the pstA domain or also encompasses the pstO domain. Overexpression of UBP1 from the *SP60* prespore-specific promoter stabilizes degradation of the F-box/WD40-GFP fusion protein in the prespore domain.

In addition to controlling proportioning of pstO and prespore cells, MEKKα appears to regulate the spatial patterning of the cell types. *mekk*α null slugs lack a defined pstO/prespore boundary and exhibit some interspersion of pstO and prespore cells. In cells overexpressing MEKKα, there is a nearly complete intermixing of prestalk and prespore cells along almost the entire length of the slug. It is unclear whether these patterning defects are regulated by the same pathway that controls the proportioning defects observed in MEKKα mutant strains. Alternatively, MEKKα may regulate other components of the pathway that controls patterning.

A second gene that regulates pstO and prespore proportioning is FbxA. A novel protein, FbxA, like MEKKα, contains an F-box and WD40 repeats at the C-terminus and, a large N-terminal domain that does not share homology with other known proteins. FbxA mutant strains have phenotypes that are the opposite of those of the equivalent MEKKα mutant strain. *fbxA* null cells exhibit an almost complete loss of the pstO domain and pstO cells and a concomitant increase in prespore cells and domain. Overexpression of FbxA results in an increase in the number of pstO cells and the size of the pstO domain and a concomitant decrease in the prespore domain (Nelson MK, Clark A, Abe T, Nomura A, Yadava N, Funair CJ, Jermyn KA, Firtel RA, and Williams JG, sub. for pub.).

The presence of the F-box and WD40 repeats suggests that FbxA is regulated by ubiquitination and degradation by the proteosome pathway and/or it regulates the degradation of other cellular proteins. It is possible that MEKKα and FbxA function on the same genetic pathway. If they do so, the present research suggests that MEKKα, a membrane-associated protein that is a putative MAP kinase pathway, may lie upstream from FbxA and negatively regulate FbxA function. FbxA could promote prestalk cell differentiation and/or inhibit prespore cell differentiation. Disruption of MEKKα, in this scenario, would cause an increase in the biological activity of the FbxA and an increase in prespore cell differentiation at the expense of pstO cells. Disruption of MEKKα could lead to an increase in the pstO domain at the expense of prespore cells. It is also possible that MEKKα and FbxA act on parallel path-

ways to differentially regulate pstO and prespore cell differentiation.

At present, it is not known how MEKKα or FbxA controls cell-type proportioning and what the upstream regulatory pathways are that regulate control by the ubiquitination pathway. Among the possible models, cAMP and/or DIF signaling could mediate the function of MEKKα and FbxA, and these proteins could modulate the proportioning pathways that control the ratios between pstO and prespore cells.

A pathway has been proposed for transdifferentiation in which pstA cells differentiate into pstO cells, which differentiate into ALCs, which differentiate into prespore cells (pstA ⇔ pstO ⇔ ALC ⇔ prespore cells). These pathways are reversible and thus pathways that control the equilibrium between ALCs and pstO cells or ALCs and prespore cells would control key steps in this process. In most strains, there appears to be little change in the ALC population, suggesting there may be other mechanisms to stabilize this population that allow pstO or prespore cells to increase or decrease proportionally. An exception may be the *ubc1* null strain. The staining pattern of *RasD / lacZ* is consistent with an increase in the ALC population.

In a few short years, we have learned a large amount about how patterning is established and genes that play key roles in controlling cell-type proportioning. In order to further understand the mechanisms controlling cell-type patterning and proportioning, elucidation of additional components of the pathways is required including the identification and function of downstream genes. As Raper's original experiments suggest that cells 'sense' the proportions of other cell types within the whole organism, future studies need to demonstrate how soluble morphogens control the activity of MEKKα, FbxA, and other components.

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