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# Breaking symmetries: regulation of *Dictyostelium* development through chemoattractant and morphogen signal-response

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*Dictyostelium discoideum* grow unicellularly, but develop as multicellular organisms. At two stages of development, their underlying symmetrical pattern of cellular organization becomes disrupted. During the formation of the multicellular aggregate, individual non-polarized cells re-organize their cytoskeletal structures to sequester specific intracellular signaling elements for activation by and directed movement within chemoattractant gradients. Subsequently, response to secreted morphogens directs undifferentiated populations to adopt different cell fates. Using a combination of cellular, biochemical and molecular approaches, workers have now begun to understand the mechanisms that permit *Dictyostelium* (and other chemotactic cells) to move directionally in shallow chemoattractant gradients and the transcriptional regulatory pathways that polarize cell-fate choice and initiate pattern formation.

## Addresses

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## Abbreviations

|              |  |
|--------------|--|
| <b>CARs</b>  | cAMP receptors                                     |
| <b>DIF-1</b> | differentiation-inducing factor-1                  |
| <b>Fz</b>    | Frizzled   |
| <b>PI3K</b>  | phosphatidylinositol 3-kinase                      |
| <b>psp</b>   | prespore   |
| <b>pst</b>   | prestalk   |
| <b>STATs</b> | signal transducers and activators of transcription |
| <b>TM</b>    | transmembrane                                      |

## Introduction

*Dictyostelium discoideum* has proven an excellent genetic and molecular system for analyzing developmental responses to both chemoattractants and morphogens. Its small (~34 Mb), sequenced genome [1<sup>••</sup>], but complex (~11,000 genes) transcriptome [1<sup>••</sup>,2] has aided research-

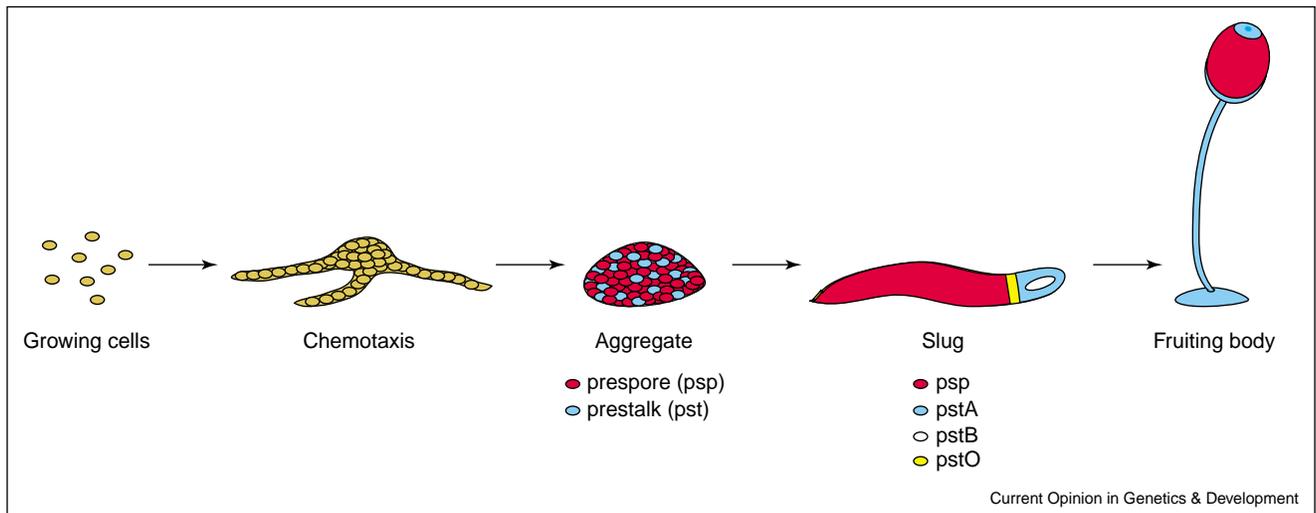
ers to dissect pathways and identify elements with universal connection throughout the metazoa. *Dictyostelium* grow as individual cells but, upon depletion of essential nutrients, initiate a highly synchronous, multicellular developmental program (Figure 1). Although development is complex, it can be characterized broadly by two distinct phases: chemotaxis and aggregation transit single growing cells to form multicellular organisms, which then undergo regulated stages of cytodifferentiation, cell sorting, and morphogenesis.

*Dictyostelium* develop on solid substrata. Upon starvation, cells within a population begin to secrete an oscillating cAMP signal. Cells respond through specific seven transmembrane (7-TM), G-protein-coupled cAMP receptors (CARs) to move chemotactically toward the cAMP source where cells aggregate into multicellular structures. Receptor stimulation also activates adenylyl cyclase for synthesis and secretion of additional cAMP that relays the wave outwardly to recruit additional cells and expand the size of the signaling territories [3<sup>••</sup>,4<sup>••</sup>]. The responses are only transient. Following activation, the chemotactic and adenylyl cyclase responses adapt to the cAMP signal. Secreted cAMP is then degraded by an extracellular phosphodiesterase, thus allowing cells to become re-responsive to the next cAMP wave. Cycling between responsive and adaptive states ensures the propagation of the cAMP signal and the inward accretion of individual cells [3<sup>••</sup>,4<sup>••</sup>].

The multicellular aggregate displays two elemental processes for cell pattern formation (Figure 1). In response to combinatorial signaling via the morphogens cAMP and DIF-1, cells initiate the differentiation of dispersed progenitor cells within the developing organism [5]. The prespore and prestalk cells, which represent the major precursors of the mature spore and stalk cells of the terminal fruiting body, then sort into specific compartments. An intermediate developmental structure, the pseudoplasmodium or migrating slug, is organized along an anteroposterior axis; the posterior (~75%) is predominantly prespore cells, whereas the prestalk cells are primarily restricted to the anterior of the slug. In turn, these compartments exhibit further subdivisions ([6–8]; see below). Eventually, the non-terminally differentiated prespore and prestalk cells give rise to the terminal spore and stalk-type cells that comprise the fully differentiated fruiting body [5].

In this review, we bridge the major response pathways that establish initial cellular patterning in *Dictyostelium*.

Figure 1



The major stages of *Dictyostelium* development. *Dictyostelium* grow as individual, undifferentiated cells. Development initiates upon starvation. Cells within the developing population secrete cAMP and surrounding cells respond chemotactically, moving inwardly toward the cAMP source where they form a multicellular aggregate. Cells within the aggregate differentiate into non-terminally differentiated prespore (red) and prestalk (blue) populations; the prestalk (pst) cells can be subclassified, but are represented in the aggregate as a homogenous population. The aggregate undergoes morphogenesis and the differentiated cells sort. At the slug stage, the posterior is primarily comprised of prespore (red) cells. Three anterior pst zones are shown; pstA cells (blue) comprise the major anterior group, the pstO population (yellow) lies between the pstA cells and the psp cells of the posterior, and pstB cells (white) lie in a central anterior core. Additional pst subclasses also exist, pstAB cells and anterior-like cells (ALCs) that populate the slug posterior. Prespore cells of the slug posterior give rise to the terminally differentiated spores (red) of the mature fruiting body. The prestalk populations differentiate into various stalk structures, illustrated as a single grouping (blue).

Recent results now begin to explain how *Dictyostelium* (and neutrophils) integrate activating and inhibitory networks to polarize and move directionally within very shallow chemoattractant gradients. We also explore the antagonistic signaling cascades that direct progenitor fate choice within aggregated *Dictyostelium* and relate these paths to that of other systems.

### Come together: chemotaxis and aggregation

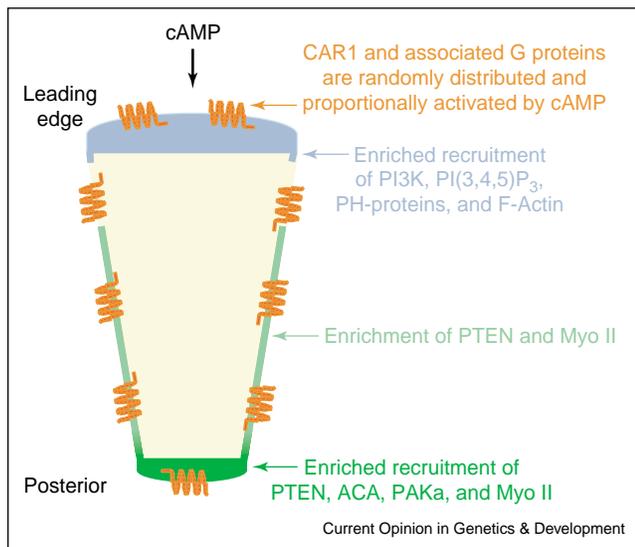
Recent studies in *Dictyostelium* have shed significant new light on how cells sense and respond to chemoattractant gradients, pathways conserved in the metazoa. Activation of CAR1, the main chemoattractant receptor, leads to the formation of a polarized, chemotaxing cell (Figure 2). Chemotaxing cells exhibit a protruding leading edge comprised of newly formed F-actin that is oriented toward the chemoattractant source, and a posterior, whose contraction is mediated by the assembly of myosin II linked to F-actin polymerization [3<sup>••</sup>,4<sup>••</sup>].

CAR1 and its coupled heterotrimeric G proteins are, in essence, distributed uniformly around the cell surface [9]. Effectors that function downstream of receptor activation also do not exhibit spatial polarity in unstimulated, non-polarized cells [3<sup>••</sup>,4<sup>••</sup>,10–13]. Upon exposure to a chemoattractant gradient, the receptors and G proteins become proportionally activated and remain uniformly

distributed [10], but many signaling components become spatially restricted to opposite poles of the cell (Figures 2 and 3). Remarkably, polarization occurs in cAMP gradients that differ by <5% across the cell body. Thus, response to the gradient induces an intracellular signaling amplification that is downstream of heterotrimeric G protein activation and that steeply polarizes the distribution of various intracellular signaling components, ultimately leading to actin polymerization at the leading edge and myosin II assembly at the posterior (see Figure 2; [3<sup>••</sup>,4<sup>••</sup>,10–13]).

One of the first (<5 s) polarized responses visualized in *Dictyostelium* cells or neutrophils is the spatially-restricted recruitment (Figures 2 and 3) and activation of phosphatidylinositol 3-kinase (PI3K) at the leading edge [13–15,16<sup>•</sup>]. By contrast, the negative regulator of the PI3K pathway, PTEN, displays an inverse pattern of localization (Figures 2 and 3). PTEN is lost from the leading edge and becomes preferentially distributed at the sides and posterior of chemotaxing cells [13,14,17,18<sup>•</sup>]. These coordinated processes serve to localize the production and accumulation of PtdIns(3,4,5)P<sub>3</sub>, which recruits a subset of PH-domain-containing proteins including CRAC, Akt/PKB and PhdA to the leading edge [19–21]. The localized actions of these proteins regulate F-actin formation and pseudopod extension at the anterior of the cell (Figure 3).

Figure 2



Differential regulation of leading edge and posterior functions mediate chemotaxis and cell polarity. *Dictyostelium* within a cAMP gradient adapt a highly polarized cellular organization. The cAMP chemoattractant receptors and their associated heterotrimeric G proteins are distributed around the cell perimeter and are activated in proportion to the cAMP stimulus. Yet, the leading edge and posterior of the cells display highly polarized distributions of specific signaling components. PI3K, PtdIns(3,4,5)P<sub>3</sub>, and the PH proteins Akt, CRAC, and PhdA preferentially localize to the edge of the cell that is closest to the cAMP source. These components direct F-actin polymerization and pseudopod extension in the direction of the gradient. Accumulation of PTEN at the sides and posterior antagonizes recruitment of PH proteins to these sites and suppresses lateral pseudopod formation. Assembly of Myosin II increases cortical tension at the sides which also restricts pseudopod extension and promotes contraction of the posterior in the direction of the cAMP source.

Cells carrying null mutations for *PI3K* have impaired polarity and chemotaxis [14]; loss of *PTEN* also leads to severe chemotaxis defects as the result of spurious PI3K signaling along the entire perimeter of the cell surface [14,17].

The rear of the cell exhibits additional polarized responses (Figures 2 and 3). The predominant adenylyl cyclase ACA and the myosin II regulator PAKa are both localized to the posterior of chemotaxing cells [22\*,23]. Interestingly, their activities are, respectively, dependent upon CRAC and Akt/PKB [23,24], signaling molecules that are localized at the leading edge [19,20]. Other data emphasize reciprocal regulatory responses between the front and rear of the cell. Cells that lack guanylyl cyclases (GCs) are defective for myosin II assembly and lose the ability to suppress lateral pseudopod formation [25,26\*\*]. As a result, these cells exhibit expanded anterior signaling. By contrast, cells carrying mutations in the cGMP-phosphodiesterases accumulate high levels of cGMP and become hyperpolarized, displaying a predominant and

narrowly restricted pseudopod at the leading edge [25,26\*\*]. Recent data in mammalian cells also indicate antagonism between the front and rear of chemotaxing cells [27–29]. However, the antagonism is not absolute. *Dictyostelium* that are simultaneously exposed to two independent cAMP gradients at opposite sides of the cell can still display polarized responses to both [13].

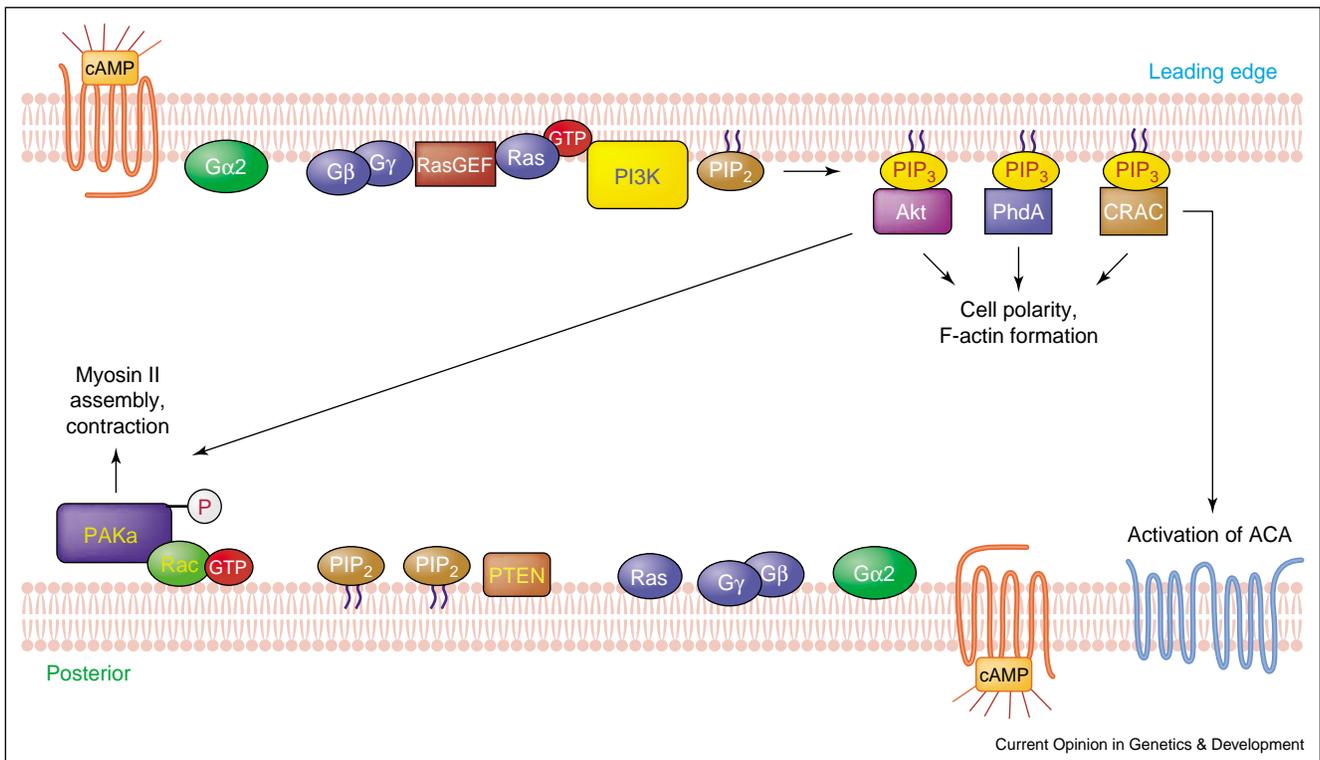
Cell polarization requires the integration of both activating and inhibiting paths to amplify shallow extracellular gradients into steep intracellular responses. Although receptor-dependent activation of Ras is suggested to be required upstream of PI3K, ACA and GC signaling [3,4\*\*,14], it is not known if Ras activation is either local or global (see Figure 3). In addition, very little is known about inhibitory or adaptive pathways during chemotaxis. In *Dictyostelium*, signaling via Gα9, a heterotrimeric G protein, regulates adaptive responses to cAMP [3\*\*,4\*\*]. But disruption of *ga9* does not eliminate adaptation or impair polarization in a cAMP gradient [26\*\*], rather *ga9*-null cells become hyperpolarized and are hypersensitive for chemotaxis [26\*\*].

### A simple twist of fate: cAMP and GSK3 signaling

Extracellular cAMP in *Dictyostelium* not only acts as a chemoattractant but also as a morphogen to regulate developmental cell choice. cAMP-signaling directs differentiation of both prespore and prestalk fates using antagonistic pathways (Figure 4). Engagement of CAR3 activates the tyrosine kinases ZAK1 [30] and ZAK2 (L Kim, J Brzostowski, AR Kimmel, unpublished) that phosphorylate two essential tyrosines in the activation loop of protein kinase GSK3 and thereby activate it [31\*\*]. Phospho-activated GSK3 drives prespore/spore differentiation but represses prestalk/stalk decisions (Figure 4). Cells that lack CAR3, ZAK1 (or ZAK2), or GSK3 or that express a kinase-inactive or non-phosphorylatable forms of GSK3 exhibit expanded expression of prestalk markers and a significant decline in spore production [30,31\*\*,32,33]. Conversely, cAMP/CAR4 activates a protein tyrosine phosphatase (PTPase) that dephosphorylates and de-activates GSK3 and stimulates prestalk differentiation [31\*\*,34]. As expected, *car4*-null cells display phenotypes largely opposite that of *gsk3*-null cells, including enhanced prespore signaling and diminished prestalk pathways [31\*\*,34].

Antagonistic regulation of GSK3 activity is central to the development of all metazoan systems (Figure 4). A primary inhibitory pathway involves response to the secreted, glycoprotein Wnt that ultimately regulates the intracellular levels of the transcriptional co-factor β-catenin (Figure 4). In unstimulated cells, β-catenin is in complex with Axin and is a substrate for phosphorylation by GSK3; phosphorylated β-catenin is then targeted for proteasomal degradation via APC and other compo-

Figure 3



Spatially restricted signaling regulates chemoattractant response. The cAMP receptors and heterotrimeric G proteins at both the leading edge and posterior of the cell are activated in proportion to the strength of the cAMP signal, but localization and activation of downstream signaling molecules are spatially restricted. At the leading edge, activation of PI3K leads to the production of PtdIns(3,4,5)P<sub>3</sub> and recruitment of the PH proteins Akt, CRAC, and PhdA. PI3K activation is presumed to require GTP-bound Ras, whose production is mediated by Gβγ activation of RasGEFs, GDP–GTP exchange factors. These latter mechanisms are predicted but have not been resolved definitively. It is also not known if Ras activation is locally restricted to the leading edge. At the posterior, there is a predominance of PtdIns(4,5)P<sub>2</sub> and PTEN which limits recruitment of PH proteins. Adenylyl cyclase ACA and PAKa localize to the posterior but require PH-domain co-activating factors. PAKa and activated Rac regulate myosin II contraction of the rear in the direction of the chemoattractant signal.

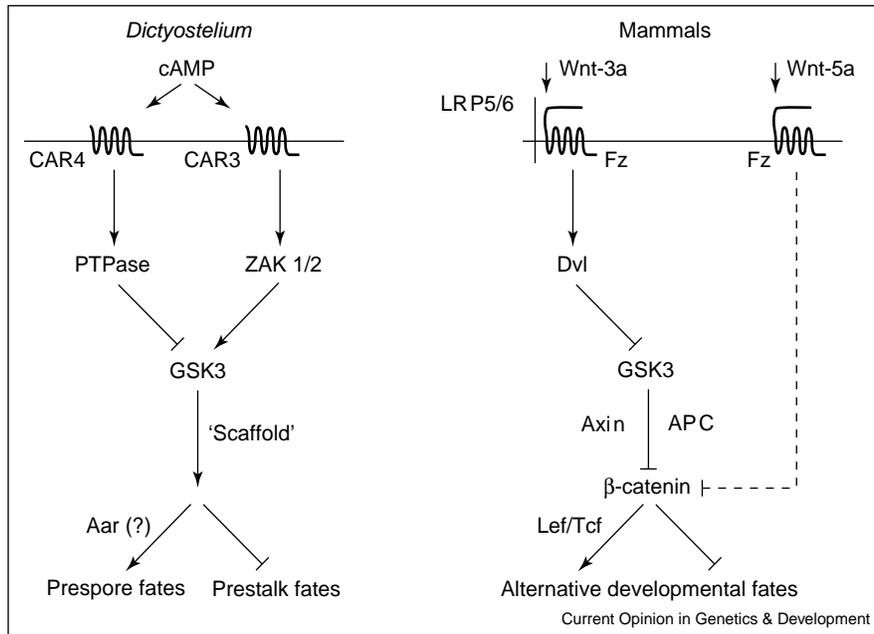
nents. In the canonical Wnt pathway, stimulation of the 7-TM Frizzled (Fz) and LRP co-receptors inhibits GSK3 phosphorylation of β-catenin by disrupting the Axin destruction complex [35]. β-catenin levels are, thus, stabilized allowing for enhanced association with the Lef/Tcf family of transcription factors. Nuclear β-catenin/Lef complexes activate gene sets that polarize specific cell-fate decisions. In vertebrates, certain Wnts (e.g. Wnt-5a) are also capable of antagonizing the canonical pathway [36]. In this context, β-catenin is the target for destabilization, but in a manner that appears to function independently of GSK3 activation (Figure 4).

Although there is no evidence in *Dictyostelium* for genes encoding Wnt-like factors, strong parallels between the cAMP/CAR and Wnt/Fz pathways are evident at several levels (Figure 4). The CAR4 and canonical Wnt pathway both transmit an inhibitory signal to GSK3, although the primary effect of Wnt-3a does not reduce GSK3 activity *per se*. Similarly, the CAR3 and Wnt-5a pathways antagonize their respective counterparts, although again

involving different mechanisms. Nonetheless, the shared, functional antagonisms directed toward GSK3 seem fundamental to multicellular differentiation and have been further underscored since first proposed [34]. A further link is apparent when the transmembrane sequences of the CAR and Fz proteins are compared (Figure 5). CARs lack the extracellular cysteine-rich, Wnt-binding domain, but the proteins share remarkable conservation despite evolutionary distances of >500,000,000 years. It should also be emphasized that in both *Dictyostelium* and the metazoa, overexpression of kinase-inactive forms of GSK3 act as dominant-negative factors to phenocopy genetic or biochemical (e.g. lithium) treatments that inhibit the endogenous activity of GSK3 [30,31<sup>••</sup>,34,35]. These data anticipated the discovery of Axin that scaffolds GSK3 and β-catenin [35], and lay a foundation for a functionally similar factor in *Dictyostelium* [30,31<sup>••</sup>].

The gene targets that lie immediately downstream of (activated or de-activated) GSK3 have yet to be identified in *Dictyostelium*. The β-catenin-related protein Aardvark

Figure 4

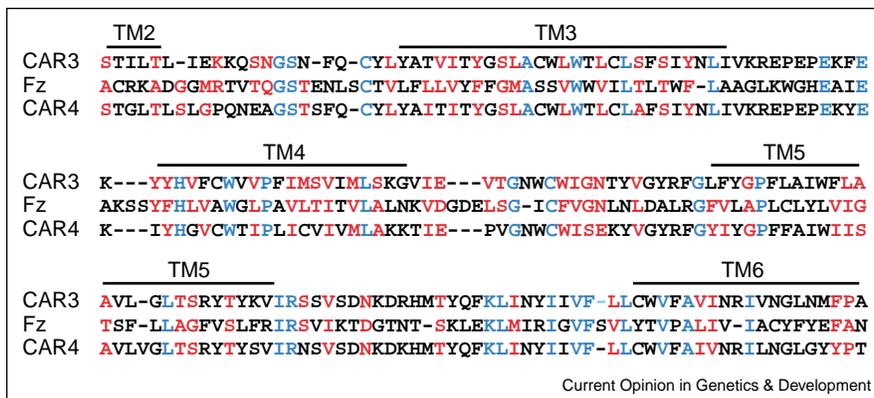


Comparative GSK3 signaling paths between *Dictyostelium* and mammalian cells. *Dictyostelium* and metazoan cells share antagonistic GSK3 signaling cascades that regulate cell-fate decisions in response to cAMP or Wnt, their respective morphogens. In *Dictyostelium*, cAMP stimulated CAR3 will activate the tyrosine kinases ZAK1 and ZAK2 that phosphorylate and consequently activate GSK3. Phospho-activated GSK3 potentially in concert with the  $\beta$ -catenin related protein Aardvark (Aar) directs prespore patterning. Prestalk fates are also inhibited by this pathway, but in an Aardvark-independent manner. CAR4 signaling antagonizes that action of GSK3 by activating a protein tyrosine phosphatase (PTPase) that de-phosphorylates and de-activates GSK3. In unstimulated mammalian cells,  $\beta$ -catenin is in complex with Axin and phosphorylated by GSK3. This promotes  $\beta$ -catenin destruction by a proteasomal pathway. Wnt-3a stimulation of the co-receptors Fz and LRP5/6 functionally inhibits  $\beta$ -catenin phosphorylation by a Dishevelled (Dvl)-dependent mechanism.  $\beta$ -catenin is thus stabilized, enhancing interaction with the Lef/Tcf family of transcription factors. Alternative signaling via Wnt-5a can de-stabilize  $\beta$ -catenin in a GSK3-independent manner.

is suggested to function downstream of GSK3 to activate prespore gene expression [37], but a definitive biochemical link has not been established. Nevertheless, this path does not appear to repress prestalk differentiation [38]

as would be predicted by analogy with the Wnt pathways. Tcf-related proteins have also not been identified. GSK3 can modulate the function of other transcription factors by regulating their nuclear localization [39,40].

Figure 5



The CAR and Fz receptors have a common transmembrane domain organization. Amino acid alignment of the central transmembrane regions of CAR3, CAR4, and the 'consensus' sequence of Fz from the NCBI domain search. Amino acid identities among the proteins are in blue, and similarities are in red. Note that the predicted transmembrane domains (TM) 2–6 of the CAR and Fz receptors also align. Only minimal sequence relationship is observed between the CAR and Fz proteins outside this region.

Potentially, CAR/GSK3 signaling in *Dictyostelium* has a significant function to regulate intracellular transport of transcription factors. Indeed, pathways for Wnt-signaling and nuclear targeting intersect during *Caenorhabditis elegans* development.

### The crossroads: DIF regulation

The differentiation-inducing factor DIF-1 is a chlorinated hexaphenone that was first purified by its ability to induce stalk cell formation in monolayer cultures of undifferentiated *Dictyostelium* cells [41,42]. In culture, DIF-1 also rapidly induces expression of gene markers specific for the major prestalk (pst) cell populations (pstA, pstB, and pstO; Figure 1) and represses prespore (psp) specific gene expression [43]. Further, DIF-1 inhibits spore-cell formation in monolayer culture. Thus, DIF-1 was considered a critical signaling molecule for establishing pattern formation. At variance, DIF-1 is synthesized in the same psp compartment it is proposed to repress [44] and is principally degraded in pst cells [45].

Des-Methyl-DIF-1 methyltransferase, DmtA, catalyzes the terminal step of DIF-1 biosynthesis. *dmtA*-null cells do not synthesize active DIF-1, but remarkably, during development they are still able to produce stalk cells normally and to induce gene markers representative of pstA and pstB cells [46]. Likewise, prespore gene expression during development is also unaffected by loss of *dmtA*. Thus, the *bona fide* function of DIF-1, as defined genetically, differs from that inferred by cell culture studies. Only one cellular compartment is altered by the loss of DIF-1; expression of certain pstO-markers, but perhaps not all [47], are absent during development of cells that lack DIF-1, indicating that DIF-1 may only function *in vivo* to promote pstO differentiation (Figure 1). Yet, *dmtA*-null cells remain responsive to DIF-1 in culture for induction of stalk and repression of spore differentiation [48\*\*]. It is still not understood why exogenous DIF-1 has such global effects on *Dictyostelium* differentiation, whereas developmental defects as a consequence of DIF-1 depletion appear to be restricted to the pstO population.

It has been difficult to identify receptors and downstream targets of DIF-1 signaling. Although, DIF-1-responsive promoter elements display high affinity for STATs (signal transducers and activators of transcription; see below), the phenotypes of cells lacking the various STATs are distinct from cells lacking DIF-1 [49\*,50,51].

To discover pathway members downstream of DIF-1, Thompson and co-workers recently screened for mutants resistant to DIF-1 inhibition of spore formation in cell culture and isolated cells that lack DimA [48\*\*], a member of the bZIP (basic/leucine zipper) family of transcription factors [52]. During development, *dimA*-null cells behave as morphological phenocopies of *dmtA*-null cells, suggest-

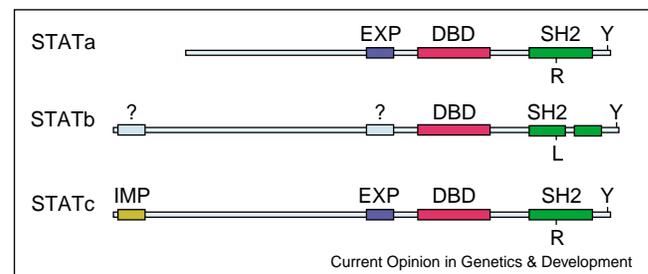
ing that DimA is required to mediate DIF-1 regulation. There are, however, significant differences between the two null lines. *dimA*-null cells produce normal levels of DIF-1 and, in culture, are completely unresponsive to exogenous DIF-1 for induction of prestalk/stalk differentiation or repression of prespore/spore fates. By contrast, cells lacking DIF-1 act non-autonomously and respond normally to the activating and inhibitory functions of DIF-1 [48\*\*].

The DimA transcription factor is required for integration of all aspects of DIF-1 response. DimA functions autonomously to mediate DIF-1 induction of pstA, pstB and pstO genes and stalk formation in culture and loss of *dimA* abrogates the repressive effects of DIF-1 on prespore/spore differentiation. It remains to be determined where DimA lies within these differentiation pathways or how DimA is able to elicit both activating and inhibitory regulation. Target genes and promoter binding sites for DimA are not known. In general, bZIP proteins function as obligate homo- and heterodimers [52–54]; potentially, the contrasting cell-specific regulations are conferred by distinct interacting partners. It is also possible that DimA is not actually in a pathway that is directly responsive to DIF-1, but is required to enable cells to respond to DIF-1.

### Here's to the STATs...

STATs are defined by three conserved elements (Figure 6): a central DNA-binding domain, a phosphotyrosine-interacting SH2 domain, and a unique tyrosine that is phosphorylated by receptor-mediated events [55]. Unphosphorylated STATs are latent, cytosolic proteins. Tyrosine phosphorylation leads to dimerization via reciprocal phosphotyrosine/SH2 interactions and nuclear translocation. Many mammalian STATs also possess a C-terminal transcriptional activation domain. Recent

Figure 6



The comparative structures of the *Dictyostelium* STATs. EXP, nuclear export domain. DBD, DNA-binding domain. SH2, SH2 (phosphotyrosine-binding) domain; the SH2 domain of STATb contains a 15 amino acid 'insertion'. R, an arginine within the SH2 domain that is essential for interaction with phosphotyrosine; the SH2 domain of STATb contains a leucine, L, at this position. Y, tyrosine phosphorylation site. IMP, nuclear import domain. ?, potential IMP- or EXP-related sequences. A fourth *Dictyostelium* STAT, STATd, may be present, but it has not been fully characterized.

discoveries in *Dictyostelium* have defined new parameters for STAT function.

STATs (Figure 6) in *Dictyostelium* were first identified through interaction with specific prestalk promoter sequences [56]. The *ecmA* gene has a complex promoter-directing expression to both pstA and pstO cells (see Figure 1). A minimal element that conveys prestalk specificity and DIF-1 inducibility contains TTGA direct repeats. *ecmB* gene expression is restricted to pstB cells and is actively repressed in pstA and pstO cells of the slug. The *ecmB* repressor contains inverse copies of the TTGA repeat and promoter constructs lacking this inverse repeat are expressed in pstA/O cells. The STATa protein was purified on the basis of its high affinity for these TTGA elements [57], and *Dictyostelium* STATc and STATb were eventually identified by cross-hybridization and bioinformatic approaches [49,51]. None contain carboxyl-terminal transactivation domains (Figure 6).

In general, STATa mediates transcriptional repression during stalk commitment [50]. However, STATa may be required to directly activate the transcription of the nuclear factor gene *CudA* in prestalk cells [57]. Although *STATa*-null cells are hypersensitive to DIF-1 and ectopically express *ecmB*, STATa is not directly regulated by DIF-1. Somewhat unexpectedly, cAMP/CAR signaling promotes the rapid tyrosine phosphorylation and nuclear translocation of STATa [58].

Like STATa, STATc functions to mediate transcriptional repression [51]. Prestalk 'A' elements within the *ecmA* promoter restrict expression to the pstA region of the slug, excluding expression from pstO cells (Figure 1). During development, STATc is selectively enriched in nuclei of pstO cells and in the absence of STATc, pstA reporters are active throughout the pstO zone [51]. The intracellular localization of STATc (Figure 6) is regulated by the balanced activities of a nuclear import element (perhaps importin  $\alpha$ 5-like) and a leptomycin-B-sensitive nuclear export domain [59]. Upon DIF-1 treatment, STATc becomes tyrosine phosphorylated leading to a dimerized state that shifts the balance toward nuclear translocation [51,59]. STATc dimerization is also activated by a stress response pathway and, although STATc lacks a transactivation domain, it functions as a transcriptional activator during hyperosmotic shock [60].

Structural aspects of STATb make it unique among STAT proteins [49]. The STATb SH2 domain contains an apparent 15 amino acid insertion (Figure 6). Nonetheless, modeling predicts this would not disrupt binding to phosphotyrosines. More significantly, the SH2 domain also lacks a basic amino acid residue that is required for interaction with phosphotyrosines. In STATb the equivalent position is a leucine (Figure 6). Interestingly, STATb is a constitutive homodimer and replacement of the

kinase-targeted tyrosine of STATb with phenylalanine does not block homodimer formation [49]. Further, STATb does not dimerize with STATc or STATa. As to biological function, STATb is localized in nuclei of all cells and *STATb*-null cells exhibit significantly impaired growth rates in comparison with parental controls [49].

Recent crystallographic analyses of *Dictyostelium* STATa have provided new insights into STAT structure in general. Dimerized mammalian STAT in complex with DNA adopts a highly compact configuration, but the structure of free STATs had not been visualized previously. Using a homodimeric core fragment of STATa, workers have now shown that STAT dimers are in a fully extended state before DNA binding [61]. Although there are structural elements of STATa that differ from mammalian STATs, these data indicate that a major structural shift must occur upon STAT interaction with DNA targets.

In the metazoan, JAKs serve as the predominant tyrosine kinase (PYK) for STAT phosphorylation [52]. The STAT-activating PYKs in *Dictyostelium* have not yet been identified. Four PYKs and three (dual-specific) SH2 domain-containing protein kinases have been characterized in *Dictyostelium* [30,31,62]. As yet, none have been linked to STAT phosphorylation.

## Conclusions and perspectives

Cell-pattern formation in *Dictyostelium* is established relatively soon after differentiation is initiated. However, none of the progenitor cells are terminally differentiated; they each retain developmental plasticity. Physical or genetic manipulations that eliminate one population of the slug can elicit the transdifferentiation between cell-types to maintain a constant prestalk/prespore ratio. Perhaps it is this essential nature of *Dictyostelium* development that underscores the extensive interplay among the signaling paths. None of the paths are exclusive or absolute. It will be the next challenge to understand how they are integrated to direct non-polarized, symmetrically organized populations of cells to establish asymmetric developmental patterns.

## Update

Recent data indicate that *Dictyostelium*, like other chemotaxing cells, exhibit a biphasic response to a uniform chemoattractant stimulus [63–65]. The initial response is rapid (~5"), but transient. Following activation of PI3K, cytosolic PH domain proteins are recruited along the entire plasma membrane [19,20,64,65]. As response to cAMP adapts, these PH domain proteins de-localize from the plasma membrane and return to the cytosol. Data now show that there is a delayed secondary translocation that persists in the presence of a continued cAMP stimulus [64,65]. The secondary translocation response of PH domain proteins is not uniform, but is restricted to distinct

patches of the plasma membrane. These targeted regions may provide spatial signals for pseudopod organization and define regulatory networks for pseudopod extension during chemotaxis.

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  - of outstanding interest
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