Dictyostelium: a model for regulated cell movement during morphogenesis

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Dictyostelium has played an important role in unraveling the pathways that control cell movement and chemotaxis. Recent studies have started to elucidate the pathways that control cell sorting, morphogenesis, and the establishment of spatial patterning in this system. In doing so, they provide new insights into how cell movements within a multicellular organism are regulated and the importance of pathways that are similar to those that regulate chemotaxis of cells on two-dimensional surfaces during aggregation.

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Abbreviations

cAR1	cAMP receptor 1
GFP	green fluorescent protein
PI3K	PI3 kinase
RLC	myosin II regulatory light chain

Introduction

Directed cell movement plays critical roles in numerous biological processes, including chemotaxis of leukocytes and *Dictyostelium* cells, metastasis of cancer cells, cell sorting during pattern formation and morphogenesis, gastrulation, movement of primordial germ cells, and migration of neural crest cells. Except for the pathways controlling chemotaxis, little is known about the mechanisms and pathways that control the directed cell movements required to establish spatial patterning of cell types and differential cell sorting. Although significant progress has been made in understanding chemotaxis of single cells, significantly less is known about the mechanisms by which directed cell movement regulates morphogenesis and the establishment of the spatially defined pattern of cell types in multicellular organisms.

The use of genetically tractable systems to examine problems in developmental biology has greatly facilitated the discovery of new pathways and molecules. In recent years, the power of classic genetic studies to associate a phenotype with a gene has been enhanced through the availability of genomic-based gene-discovery methods and refinement of protein–protein interaction-based methods to find additional components of a pathway. The slime-mold *Dictyostelium* is a genetically tractable system that is also amenable to the biochemical and cell biological methods necessary to dissect and understand the regulatory pathways controlling morphogenetic movement. For example, the ability to follow, *in vivo*, the morphogenetic movements of mutant cells within the context of a wild-type organism adds to our understanding of the mutant phenotypes. In this review, we examine some of the recent findings in the analysis of cell movement in *Dictyostelium*. Not unexpectedly, many of the gene products required for cell sorting within the multicellular aggregate, the establishment of spatial patterning, and morphogenesis are components of the pathways that control chemotaxis. In the same way that analysis of chemotaxis in *Dictyostelium* provides understanding of similar pathways in mammalian cells, lessons learned about cell sorting and morphogenesis in *Dictyostelium* may provide new insights into the regulatory pathways controlling these processes in metazoans.

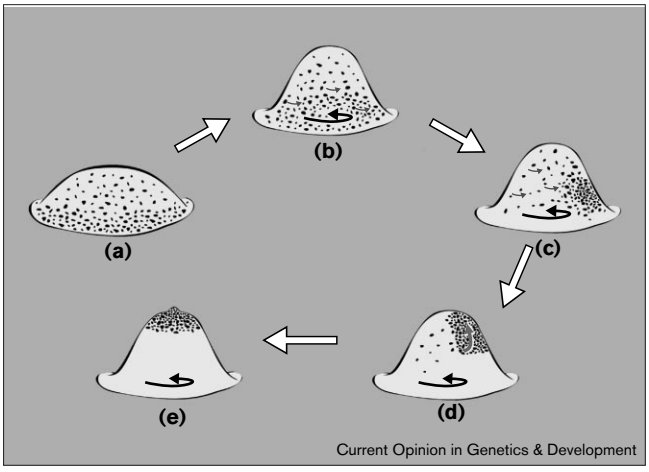
Dictyostelium as an experimental system for morphogenesis

Dictyostelium grows and divides as a single-cell amoebae. Upon starvation, up to 10^5 cells aggregate to form a multicellular organism in ~8 hours. As the mound forms, two primary cell types — prestalk and prespore — differentiate. The initial stages of morphogenesis involve the formation of an apical tip after movement of the prestalk cells to the apex of the mound. This prestalk domain elongates to form a first finger. By that time, the anterior–posterior axis is established with the prestalk cells at the front and the prespore cells at the rear. The finger falls over to form a migrating slug, or pseudoplasmodium, that is both phototactic and thermotactic. In response to environmental signals, the slug undergoes culmination, resulting in the formation of a mature fruiting body containing terminally differentiated spores and stalk cells (for review, see [1]).

cAMP as a chemoattractant regulating aggregation

Because the signaling pathways controlling aggregation in *Dictyostelium* have been recently reviewed in depth [1–3], we simply outline the features of the pathway relevant to morphogenetic movement. Aggregation is mediated by chemotaxis to cAMP. A cAMP signal, initiated at the aggregation center, is relayed outward through a field of cells as a wave. cAMP emitted from one cell binds to the cell surface, G-protein-coupled cAMP receptors (cAR1) on adjacent cells, activating adenylyl cyclase and thus producing more cAMP and relaying the signal further from the aggregation center. Additionally, cells respond by chemotaxing in towards the aggregation center. The signal is oscillatory, with a 6-minute periodicity, subdividable into an activation/response phase of ~1 minute followed by adaptation of the pathways for 5 minutes, during which time the cells are unresponsive to cAMP stimulation. The cells become resensitized after the degradation of extracellular

Figure 1



Cartoon of the cell sorting and the initial spatial patterning in *Dictyostelium*. The present models of the initial stages of morphogenesis after aggregation and formation of the multicellular organism in *Dictyostelium* are illustrated. **(a)** Prestalk cells are found scattered throughout the organism with a higher concentration around the periphery of the developing aggregate [11]. **(b)** After aggregation is completed and the tight aggregate is formed, the cells within the aggregate rotate [5]. **(c)** Prestalk cells start to move by the chemoattractant-mediated chemotaxis of the prestalk cells toward the central, basal region of the mound [4••]. **(d)** The cluster of prestalk cells then migrates upward (gray arrow), **(e)** forming the apical tip. Evidence indicates that the initial cluster formation and its movement upward to form the tip are mediated chemotactically using cAMP as the chemoattractant signal (see [4••] for details).

cAMP by a secreted, membrane-associated phosphodiesterase. The presence of an adapative period is an essential part of the directionality of cell movement towards the aggregation center during chemotaxis.

The formation of the apical tip, the initial stage in morphogenesis and cell patterning require differential chemotaxis

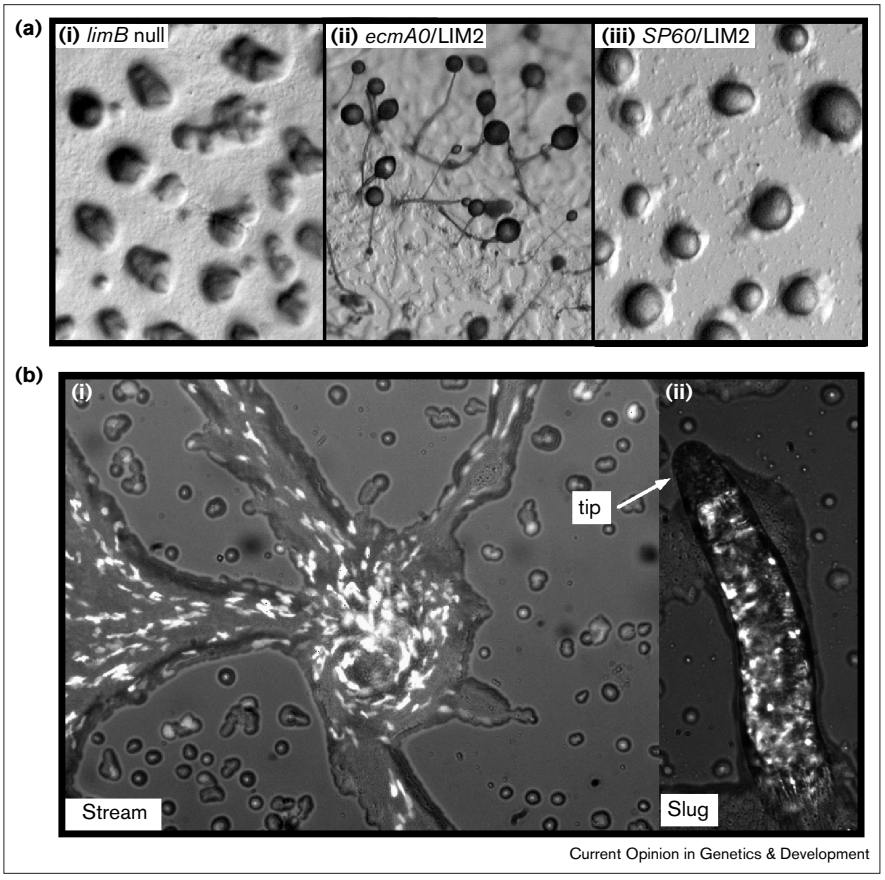
New insights into the morphogenetic movements of prespore and prestalk cells and the underlying mechanisms resulting in the formation of the apical tip and the establishment of axial polarity have come from the use of time-lapse video microscopy to record the movements of prestalk cells that were tagged with green fluorescent protein (GFP). These studies show that, after aggregation, prestalk cells (cells expressing GFP under the control of a prestalk-specific promoter) move inward towards the center of the mound, forming a cluster of prestalk cells [4••] (Figure 1). Depending on the strain used, a general rotary movement of cells within the mound might overlay the inward movement [4••,5]. After clustering near the base in the center of the mound, the cells move upward to form the tip. Analysis of the trajectories of individual cells suggests that the inward and upward movement of the prestalk cells is highly directional and may be chemotactically driven rather than being the result of differential cell adhesion, another model put forth for cell sorting [6]. The data supporting the chemotaxis-based cell-sorting model, however, do not rule out a partial contribution of differential cell adhesion.

Other studies correlating cell-shape changes and cAMP levels during morphogenesis further support the model that cell sorting is mediated by chemotaxis to cAMP, with the apex of the mound having a function similar to the aggregation center initiating cAMP waves and serving as the organizing center for the slug [7]. These waves can be observed as optical wave patterns by dark-field microscopy, representing changes in cell shape and movement in response to cAMP. The waves move outward from the tip in the form of three-dimensional scrolls [8]. Consistent with these optical waves resulting from underlying cAMP waves, the sorting of the prestalk cells and the later scroll waves in the slug are sensitive to inhibitors of adenylyl cyclase and the misregulation of the extracellular phosphodiesterase, which degrades the chemoattractant signal [9]. Moreover, placement of cAMP in the substratum beneath a mound results in prestalk cells moving downwards through the mound (see [1] for review). The idea that cAMP waves serve directly as the organizer for morphogenesis is further supported by experiments demonstrating that cAMP oscillations emitted from a micropipette placed in a slug, thus mimicking or replacing the apical organizer, cause the preferential migration and accumulation of prestalk cells at the cAMP source [10].

The conclusion that the prestalk cells actively sort from the prespore cells is in agreement with experiments demonstrating that the prestalk A cells — the subclass of prestalk cells found at the apex of the first finger and the front of the slug — move with the highest speed, followed by the prestalk O cells, which locate to a band immediately posterior to the prestalk A domain [11]. The prespore cells, which are found in the posterior of the organism, have the slowest rate of movement. This is consistent with axial patterning being established through the differential rates of chemotactic movement directed by cAMP waves that are initiated at the tip of the developing slug.

If chemotactic sorting of prestalk cells is essential for morphogenesis in *Dictyostelium*, a defect in tip formation resulting from a gene knockout might be complemented by the prestalk-specific re-expression of this protein, whereas expressing it exclusively in prespore cells might not. Such a cell-type-specific complementation has been demonstrated for two proteins that are required for proper chemotaxis and morphogenesis. One is the myosin II regulatory light chain (RLC), which is required for the regulation of conventional myosin (myosin II) [4••,12], and the second is LIM2, a LIM-domain-containing protein required for proper reorganization of the actin cytoskeleton [13•] (Figure 2a). These experiments support involvement of the sorting of prestalk cells in the morphogenetic process and suggest that prespore cells are mostly passive in the process of morphogenesis, at least at these stages of development.

Figure 2



Morphogenesis and patterning. **(a)** Illustration of the role of prestalk cells controlling morphogenesis in *Dictyostelium*. **(i)** The terminal phenotype of a gene knockout of *limB*, which encodes the LIM-domain-containing protein LIM2. The cells arrest at the mound stage. **(ii)** When LIM2 is preferentially expressed in prestalk cells using a prestalk-specific promoter, it complements the null phenotype, producing normal fruiting bodies. **(iii)** When LIM2 is expressed from a prespore-specific promoter, the null phenotype is not complemented (from [13•]; see reference for details). **(bi)** Formation of a developing aggregate with the aggregation streams and formation of the mound. The aggregate is a chimera containing predominantly wild-type cells (unlabeled) and *pkbr1* null cells tagged with GFP. Although *pkbr1* null cells aggregate normally, as can be seen in (i), they are unable to populate the anterior prestalk region (absence from the anterior of the slug **[ii]**). This is not caused by an inability to express prestalk-specific genes but is thought to be caused by a defect in cell movement. *pkbr1* null cells, when developed by themselves, arrest at the mound stage (from [27•]; see reference for details). The developing organisms are visualized by mixing some visible light with the ultraviolet to show the nonfluorescent cells more clearly.

Mutations that affect chemotaxis and morphogenesis

Cell movement during chemotaxis involves the polarization or differential subcellular localization of components of the actin/myosin cytoskeletons [14,15]. In response to directional signals, cells produce a pseudopod or lamellipod at the leading edge of the cell while the rear cell body contracts. Polymerization of G-actin monomers to form an F-actin filament in the direction of the chemoattractant source. The F-actin filaments impinge on the plasma membrane to form a pseudopod. In wild-type cells, the vast majority of pseudopodia are produced only in the direction of the chemoattractant source. There appear to be inhibitory mechanisms to prevent lateral pseudopod formation, which would result in a change in direction of cell movement. The posterior contraction is mediated by myosin II (conventional myosin) enabling the rear of the cell to free itself and lift off the substratum to move in concert with the leading edge [16]. In addition, myosin II assembly provides the needed cortical tension to give the back and sides of the cell some rigidity, which probably helps restrict pseudopod extension on these surfaces [16,17].

Not unexpectedly, cell movement during morphogenesis in *Dictyostelium* involves many of the same pathways required for aggregation. Strains carrying temperature-sensitive mutations in the sole Gβ subunit or the MAP kinase ERK2, two proteins required to regulate cAMP levels in aggregating cells, are blocked in morphogenesis when shifted to a non-permissive temperature [18,19]. Mutant strains that exhibit cytoskeletal defects and defects in chemotaxis during aggregation often exhibit defects during morphogenesis as well [20]. Although chemotaxis is not normal in the case of *myosin II* or *RLC* null cells, the cells aggregate to form a mound, but subsequent morphogenesis is blocked, and development arrests at the mound stage (see [4••]). Table 1 lists known genes that affect morphogenesis.

On the other hand, genetic screens for strains that are unable to aggregate or undergo morphogenesis identified genes required for proper chemotaxis and morphogenesis. The affected proteins fall into several classes: proteins that are part of the basic machinery required for cell motility (e.g. myosin II), proteins required for production or detection of cAMP and the immediate downstream pathway, or proteins required for the differentiation of prestalk cells [1,13•]. Using molecular and biochemical assays for gene expression and activation of adenylyl cyclase, and *in vitro* single-cell assays for cell movement, the pathways affected can be readily identified. Most strains that are blocked in the ability to produce cAMP can respond to cAMP and chemotax towards a micropipette containing cAMP

Table 1

Proteins thought to be required for morphogenesis in *Dictyostelium*.

Protein	Allele	Multicellular phenotype	Reference
α-actinin	Knockout	Arrest at the mound stage	[28]
ERK1/PTP2 (MAP kinase/ protein tyrosine phosphatase)	ERK1 overexpression in PTP2 null	Abnormal morphogenesis	[29]
IQGAP	Knockout	Abnormal morphogenesis	[30,31]
Myosin II heavy chain	Knockout	Arrest at the mound stage	[21]
Myosin II heavy chain kinase	Knockout	Arrest at the mound stage	[32]
Myosin essential light chain (ELC)	Antisense	Arrest at the mound stage	[33]
Myosin regulatory light chain (RLC)	Knockout	Arrest at the mound stage	[34]
PAKa (p21-activated Ser/Thr kinase)	Knockout	Delayed morphogenesis	[17]
PTP3 (protein tyrosine phosphatase)	Overexpression	Arrest at the mound stage	[35]
SCAR (related to WASP)	Knockout	Multiple tips	[36]
Talin homologue talB	Knockout	Morphogenesis blocked	[37]
Rac1 (small GTPase)	Dominant negative/constitutively active	Delayed development; developmental arrest	[22]
ERK2 (MAP kinase)	Temperature-sensitive mutant	Developmental arrest at the non-permissive temperature	[18]
Gb subunit (heterotrimeric G protein sub.)	Temperature-sensitive mutant	Developmental arrest at the non-permissive temperature	[19]
LIM2 (LIM domain protein)	Knockout	Arrest at the mound stage	[13•]
cAR2 (cAMP receptor)	Knockout	Arrest at the mound stage	[38]
PKBR-1 (Akt/PKB-related)	Knockout	Arrest at the mound stage	[27•]

Proteins listed in the table are thought to have a direct role in controlling morphogenesis. Some of the proteins may also have an effect on patterning through a regulatory role in controlling gene expression.

[1,2,15]. Conversely, many strains that are defective in chemotaxis exhibit normal activation of adenylyl cyclase. Some genes are required for both processes. In strains that arrest at the mound stage, one can determine whether the defect is cell-autonomous or non-autonomous and whether the mutation affects cell motility, cell-type differentiation, or production of cAMP or another chemoattractant.

A powerful approach for this characterization is the use of chimeras. In *Dictyostelium*, chimeras are readily produced by mixing cells of different genetic backgrounds and allowing them to co-aggregate to form a chimeric mound. Using GFP (or *lacZ*) reporters expressed from constitutive or cell-type-specific reporters, cells can be tagged and, in the case of GFP, visualized *in vivo* during morphogenesis. Mutant cells may co-aggregate with wild-type or other mutant cells but are often unable to sort within a wild-type mound and thus do not form part of the anterior, prestalk region, even though they are fully competent to differentiate into prestalk cells (Figure 2b). The inability to participate in the formation of the tip may be as a result of a defect in chemotaxis. As prestalk cells are more chemotactically responsive and move faster than prespore cells [11], cells that cannot move fast or cannot recognize directional cues within the developing tip are excluded from the tip [1,4••,12,13•]. As cell-type differentiation is very plastic in *Dictyostelium* until culmination, mutant prestalk cells that do not reach the anterior can dedifferentiate and then differentiate into prespore cells.

As mentioned above, some morphogenetic mutants that were identified by their inability to develop past the mound stage exhibit chemotaxis defects during aggregation. These strains aggregate but do so inefficiently (see [12,13•]). Detailed analysis of chemotaxis reveals that the strains exhibit defects in the ability to properly polarize and/or move and often have altered organization of the actin/myosin cytoskeleton. Complementary studies on null strains produced by knockout technology of genes that encode components of the cytoskeleton identified by biochemical methods demonstrate that such strains also exhibit chemotactic and morphogenetic defects ([20]; Table 1). *Myosin II* or the *RLC* null cells aggregate fairly normally but arrest at the mound stage [12,20,21]. More detailed analysis reveals further differences in the morphogenetic defects between strains. For example, *RLC* null cells initiate the sorting process and start to form prestalk clusters, albeit inefficiently, but cannot migrate to the apex [4••,12]. In contrast, null cells of the LIM domain protein LIM2, which have a defect in the actin cytoskeleton, exhibit a more severe defect and are blocked earlier in this process [13•]. These cells co-aggregate with wild-type cells but are unable to penetrate the mound and are excluded from tip formation much earlier than *RLC* cells. Similarly, strains expressing dominant negative Rac1, which is involved in regulating chemoattractant-stimulated actin polymerization and myosin assembly through the regulation of PAKa, participate in mound formation but localize to the posterior of the slug, presumably because of motility defects [17,22]. Other cytoskeletal proteins or proteins that control the cytoskeleton have been demonstrated to be important for morphogenesis, although in some cases a detailed analysis of their role in multicellular development has not yet been undertaken (see Table 1).

Why do many mutants exhibit a stronger aberrant phenotype during morphogenesis than aggregation? A major difference in *Dictyostelium* between aggregation and morphogenesis is that aggregation involves chemotaxis on a two-dimensional surface, whereas morphogenesis involves chemotaxis and cell movement in between other cells within a three-dimensional mass. Mutations that affect myosin assembly (Table 1) are expected to have reduced cortical tension and may have insufficient 'rigidity' to push their way between tightly packed cells. Movement through a cell mass is thus more demanding on mechanical properties and regulation of the cytoskeleton than movement on a two-dimensional surface in which cells do not have to push other cells aside.

Regulating polarity and directional movement

Imaging of GFP-tagged components of the chemotaxis pathway in living cells, such as receptors, PH domain-containing proteins, Gβ subunits, as well as cytoskeletal components, has made it possible to follow changes in the subcellular localization of proteins in response to chemoattractant signals and deepened our understanding of how the proteins cooperate to produce directional movement [3,14,23,24•]. Migratory or chemotaxing cells are highly polarized, with F-actin preferentially found at the front and some at the posterior of the cell. Additionally, in chemotaxing *Dictyostelium* cells, myosin II is highly localized in the posterior (see [15] for review). Although many migratory cells have a stable intrinsic polarity, this polarity can be disrupted and cells can establish a new polarity axis in response to changing external signals such as a chemoattractant gradient. Recent studies suggest that when cells are placed in a chemoattractant gradient, a localized activation of responses at the leading edge may play an important part in establishing and aligning the cells' polarity. In polarized *Dictyostelium* cells, the cAMP receptors remain uniformly distributed on the plasma membrane [23]; however, a gradient of Gβγ subunits that is highest at the leading edge is observed once the cell polarity is established [25]. This may aid cells in producing a stronger response at the leading edge and be part of the mechanism responsible for establishing and maintaining a distinct leading edge in a shallow chemoattractant gradient existing under normal *in vivo* conditions.

GFP tagging of the PH-domain-containing protein CRAC, which is essential for adenylyl cyclase activation [3], and the PH domain from Akt/PKB, a conserved serine/threonine protein kinase required for cell polarity and proper chemotaxis [24•], reveals that they rapidly and transiently translocate to the plasma membrane when cells are bathed in the chemoattractant cAMP. In chemotaxing cells, a distinct localization to the leading edge can be seen. Similar observations were recently made in neutrophils for the PH domain of mammalian Akt [26], suggesting that this pathway is conserved between *Dictyostelium* and man. Studies using null mutations of PI3 kinase (PI3K) or inhibitors of PI3K indicate that these translocations probably result from the binding of the PH domains to $PI(3,4,5)P_3$ or $PI(3,4)P_2$ generated at the leading edge, presumably through the activation of PI3K [24•].

Studies of *akt/pkb* null cells indicate that Akt/PKB activation is required for proper cell polarization and chemotactic movement [24•]. *akt/pkb* null cells do not elongate but instead produce multiple pseudopodia simultaneously around the cell. Movement is still directed towards the chemoattractant source, however, indicating that overall pseudopod protrusion must be biased in the direction of the source. Evidence that simple Akt/PKB activation is insufficient to establish polarity derives from experiments using a strain that expresses a mutant Akt/PKB with an amino-terminal myristoylation site which results in its constitutive localization along the entire plasma membrane. This localization results in a constitutive activation of Akt/PKB but such cells exhibit chemotaxis defects similar to those of *akt/pkb* null cells, possibly because Akt/PKB is not activated at the proper site in the cell. These results indicate that localized activation of Akt/PKB at the leading edge is essential for proper cell polarity and chemotaxis.

Akt/PKB protein is present during growth and aggregation, but the protein is no longer expressed after mound formation and thus cannot be required for morphogenesis. Recent evidence, however, implicates a novel Akt-related protein, PKBR-1, in morphogenesis [27•]. PKBR-1 and Akt/PKB have highly related kinase domains and carboxy-terminal extensions, and the regulatory phosphorylation sites of Akt/PKB that are required for activation in response to chemoattractant stimulation are also found in PKBR-1. Instead of an amino-terminal PH domain, however, PKBR-1 has a myristoylation site, and the protein is constitutively localized at the plasma membrane. Unlike the myristoylated Akt/PKB mutant [24•], PKBR-1 is not constitutively active, presumably because the amino-terminal domain of the protein or possibly another protein holds the kinase in an inactive state. PKBR-1 is expressed early in development and is induced at the mound stage. The PKBR-1 protein is initially present in all cells but during tip formation it is exclusively expressed in those cells that will populate the tip. Development of a *pkbr-1* null strain essentially stops at the mound stage and the few organisms that continue to differentiate produce abnormal fruiting bodies. Studies performed with chimeric organisms reveal that *pkbr-1* null cells are unable to populate the anterior prestalk domain and are predominantly localized towards the posterior of the slug — defects that suggest a cell-motility defect. This observation extends the obvious structural relatedness between Akt/PKB and PKBR-1 to the functional level, a model that is supported by the observations that expression of Akt/PKB during the multicellular stages complements the *pkbr-1* null phenotype. This complementation also leads to the expectation that some of the regulation of cell motility by these two kinases is mediated by common substrates. Interestingly, a restricted localization of PKBR-1 to the leading edge does

not appear to be required for its function, although PKBR-1 kinase activity may be preferentially activated there. Thus, *Dictyostelium* has evolved a second Akt/PKB gene to control morphogenetic movement.

It is unclear why there are two different modes of membrane localization for proteins that, at least during aggregation, have overlapping functions. Membrane localization is essential for the activation and function of both proteins. Differences in the signals between cells during aggregation and morphogenesis could presumably account for the differences between Akt/PKB and PKBR-1. During aggregation, at least in the initial stages, cells are separated; during morphogenesis, cells are in a multicellular mass and receive cell–cell contact signals as well as long-range directional chemoattractant signals.

Conclusions

A key to understanding morphogenesis will be the definition and dissection of the signaling pathways that establish cell polarity and lead to the directional activation of F-actin polymerization at the front of cells. Soluble ligands and signals associated with the extracellular matrix are expected to be important in directing the morphogenetic movements necessary to produce a complex organism and allow cells to move into appropriate positions for subsequent signaling pathways which regulate cell-fate decisions. The basic pathways being elucidated in *Dictyostelium* contribute to understanding these processes in more complex systems.

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