Chemotaxis: finding the way forward with Dictyostelium

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Understanding cell migration is centrally important to modern cell biology. However, despite years of study, progress has been hindered by experimental limitations and the complexity of the process. This has led to the popularity of Dictyostelium discoideum, with its experimentally-friendly lifestyle and small, haploid genome, as a tool to dissect the pathways involved in migration. This humble amoeba is now established at the centre of dramatic changes in our understanding of cell movement. In this review we describe the recent reinterpretation of the role of phosphatidylinositol trisphosphate (PIP3) and other intracellular messengers that connect signalling and migration, and the transition to models of chemotaxis driven by multiple, intertwined signalling pathways. In shallow gradients, pseudopods are generated with random directions, and we discuss how chemotaxis can operate by biasing this process. Overall we describe how Dictyostelium has the potential to unlock many fundamental questions in the cell motility field.

Introduction

All eukaryotes depend on cell motility at some point in their life cycle, and the ability to migrate, adhere, and change shape is of fundamental importance to a wide range of basic cellular processes. Cell motility is most commonly studied in higher eukaryotes where it is essential for cell division and embryogenesis, as well as for wound-healing, the hunting and killing of pathogens by immune cells, and for a host of other aspects of cell physiology. While a few cells respond primarily to internal cues, for example during cytokinesis or to change shape, most need to move in response to external signals. One of the most interesting and important responses is chemotaxis, where cells move towards (or occasionally away from) a diffusible chemical signal.

Cell migration is a complex process, or to be more accurate, a complex set of interacting processes. Cells must solve a number of issues in order to move towards an attractant source. They must detect the attractant, often present as only a small number of molecules, against a background of other signals; they must transmit the information to the motile machinery inside the cell, and they must somehow extract and integrate the information about the source’s direction so as to migrate in a co-ordinated fashion up the attractant gradient. Alteration of any of these processes leads to changes in the chemotactic behaviour of cells. Mostly this leads to diminished chemotaxis, but inappropriate gains in chemotaxis are also harmful, for example during cancer metastasis, when cancer cells migrate into the circulation and escape from the primary tumour to colonise other sites [1]. The development of secondary tumours is often the most difficult aspect of cancer to treat and has led to increased interest in the study of cell migration as a target for anti-metastatic therapy.

Although the importance of cell migration in mammalian cells is obvious, it is often difficult to address simple questions within the complexity of a whole organism. Even in tissue culture, changes in many of the processes underlying chemotaxis often lead to secondary effects such as apoptosis. This has led to the use of several alternative organisms to study the basic underlying principles and mechanisms behind cell movement. Prominent amongst these has been the genetically tractable social amoeba Dictyostelium discoideum; this organism has allowed us to ask fundamental questions about the biology of cell migration in a relatively controlled and amenable way. The genetics of Dictyostelium has been developed for over 50 years and genetic approaches have been used to study a number of processes. Because the lifestyle of Dictyostelium is totally dependent on efficient motility, this organism is particularly suited for the study of cell movement.

Dictyostelium lives in the soil, catching and eating bacteria. This requires the cells to be constitutively motile, and they are able to move towards folate secreted by bacteria and to move rapidly, in a manner similar to some cells of the mammalian immune system (Box 1) [2]. In addition, Dictyostelium also has a developmental cycle that is initiated in order to survive starvation, and which relies heavily upon chemotaxis. During Dictyostelium development, starving cells begin to secrete waves of cyclic adenosine monophosphate (cAMP) that acts as a chemoattractant for neighbouring cells; these then rapidly move towards each other to form aggregates of tens or hundreds of thousands of cells (Box 1). These aggregates subsequently differentiate into a number of distinct cell types, eventually resulting in the formation of a fruiting body consisting of a ball of spore cells held up by a stalk so that they can be dispersed and find a new food supply.

There are a number of reasons why Dictyostelium is an attractive organism for basic research, but by far the biggest is the ease with which it can be grown and genetically manipulated. Dictyostelium cells are haploid and therefore it is relatively simple to disrupt genes and generate a knockout of a gene of interest as well as to generate libraries of mutants for genetic screens. In addition, the Dictyostelium genome is relatively small and compact,
Box 1. Migration in *Dictyostelium* and neutrophils compared

*Dictyostelium* and neutrophils share many of the same challenges and have similar characteristics. Whereas neutrophils hunt down bacteria in the bloodstream moving towards inflammatory factors and other factors secreted by the bacterium, *Dictyostelium* performs a similar task in the soil (Figure Ia,b). *Dictyostelium* also has a second use for chemotaxis, when individual cells starve and stream together to form a multicellular aggregate, the stage at which they are most commonly studied. Here they attract each other by secreting cAMP from the rear, causing long chains of cells to form as they stream towards a central mound and differentiate (Figure Ic).

In *Dictyostelium*, neutrophils, and most motile cells, migration is achieved by generating cellular polarity, extending actin-rich structures known as pseudopods at the front, while retracting the back using myosin II (see Figure I). It is the regulation of these processes, and how they are directed by chemoattractant receptor activation, that is the key to the understanding of directed migration.

**The generation of pseudopods**

The general model of eukaryotic cell migration is that the motile force is generated by the local polymerisation of actin at the leading edge – pushing the membrane forward and generating protrusions (generally known as pseudopods; mammalian cells frequently use a specialised subset of flat, sheet-like pseudopods named lamellipodia) [3]. In general this process involves the nucleation of new actin filaments mediated by the Arp2/3 complex [4]. The Arp2/3 complex itself is strongly activated by the proteins of the Wiskott-Aldrich syndrome protein (WASP) family [5]. Members of this family serve different roles within the cell and generate different actin structures, but SCAR (also known as WAVE) is the most predominant in the generation of pseudopods and lamellipodia, localising in a thin line at the very leading edge of such protrusions [6–8]. SCAR/WAVE is therefore crucial for movement and for the maintenance of cell shape [9].

The phenotypic effects of disrupting SCAR in *Dictyostelium* are subtle. SCAR is a member of a conserved complex, with at least 4 other members, that are all required for the proper regulation of chemotaxis [9,10]. Disruption of each of the members causes defects in actin organisation, with either hypergeneration and persistence of pseudopods or a lack of large protrusions [11–13], indicating that SCAR is part of the dominant mechanism for generating pseudopods.

Surprisingly, however, *Dictyostelium* cells lacking SCAR remain capable of chemotaxis, and move with only a relatively small reduction in speed [11,14]. Therefore *Dictyostelium* cells must be able to utilise other, SCAR-independent, mechanisms to move. This exemplifies the diversity of behaviours that *Dictyostelium* displays. In contrast to more specialized cells, such as fish keratocytes that move using a singular mechanism (in this case extension of a single, huge lamellipodium), *Dictyostelium* cells exhibit a versatile range of motile behaviours, and simultaneously produce filopodial spikes and pseudopods, as well as membrane blebs (Box 2) [15]. Whereas this undoubtedly complicates matters, it can also be an experimental advantage as we can measure the relative contributions of each of these behaviours to overall cell movement.

**Signalling responses to chemoattractants**

In order to move towards a chemical signal the cell must read the extracellular attractant gradient and transmit
The evolving story of PIP₃
A popular hypothesis is the idea that the cell has some sort of chemical ‘compass’, strongly orientated in the direction of the chemoattractant, that locally induces actin polymerisation and the production of new pseudopods at the part of the cell closest the signal [18]. The first molecule to be identified that fitted the bill as the needle of an internal compass was phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), a molecule that recruits certain proteins containing a specific type of PH domain to the plasma membrane. PIP₃ is produced by type I phosphatidylinositol (4,5)-bisphosphate 3-kinases (PI3Ks) in response to extracellular stimuli, and is strongly enriched at the leading edge of both Dictyostelium and migrating neutrophils in strong gradients of chemoattractant [17,19–23].

The localisation of PIP₃-binding proteins in Dictyostelium can reorientate rapidly when the direction of the gradient is changed, coinciding with the new site of actin polymerisation [24]. Furthermore, if the PI3K 5-phosphatase PTEN is disrupted, the region of the membrane producing PIP₃ is broadened as is the region in which pseudopods are produced [25]. Therefore it seemed that the production of PIP₃ is sufficient to stimulate the production of new pseudopods and it was proposed that this was the major factor determining the directionality of the cell [18,26].

Recently, however, the notion that PIP₃ acts as a singular ‘compass’ to steer the cell has had to be reassessed. Although pharmacological inhibition and genetic disruption of PI3K has been shown repeatedly to reduce the chemotactic efficiency of both Dictyostelium and mammalian cells [23,27–30], Dictyostelium cells lacking all 5 type I PI3-kinase genes, and therefore unable to generate any PIP₃, are still capable of chemotaxis in steep gradients [31]. Therefore, while it plays an important role in maintaining efficient migration, the generation of PIP₃ cannot be the sole factor determining directionality.

What does and doesn’t PIP₃ do?
The realisation that PIP₃ is not essential for chemotaxis has led to reassessment of its exact role in migration. Careful observation of the effects of PI3K inhibitors has indicated that the localisation and functional role of PIP₃ may differ depending on how strong a signal the cell experiences. For example, the strong localisation of PIP₃ at the leading edge is actually seen only in steep gradients and at cell-cell junctions [32]; when the signal is much weaker PIP₃ is almost uniformly distributed and faintly, if at all, enriched at the leading edge (Figure 1) [33]. PIP₃ can be strongly localised when a weak cAMP signal is applied globally, but is restricted to randomly distributed, self-organising patches that coincide with pseudopod extension, indicating that PIP₃ can in some circumstances sensitise the pseudopodium-generating machinery [24,34].

The self-organising patches suggest a mechanism involving multiple feedback loops, such as those proposed by Gierer and Meinhardt [35], that can allow small local inhomogeneities to establish stable patterns of polarity. This suggests that the accumulation of PIP₃ is complex and that the feedback loops regulating PIP₃ signalling are modulated by external signal strength. Furthermore,

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**Box 2. Membrane blebbing**

One of the most interesting, but poorly understood, aspects of cell migration is the contribution of blebbing. Membrane blebs are protrusions in which the membrane is forced outwards by the internal hydrostatic pressure of the cell, ripping it away from the cortical cytoskeleton and causing a bulbous projection (for a review see Ref. [72]). The force for this is generated by myosin II contraction (in the same way that squeezing a partially inflated balloon causes an unrestrained region to bulge out), but it is unclear whether the position in which the bleb forms is determined either by local disruption of cortical actin or by localized build-up of pressure [73]. Blebbing is a well-documented phenomenon that occurs in a wide range of cell types [74], but has recently gained interest following the observation that cancer cells are able to switch between motility driven by actin polymerization at the leading edge, and blebbing-driven motility, to invade into 3D matrices [75,76]. It has become common in the field to refer to the former as ‘mesenchymal’ motility, and the bleb-based mechanism as ‘amoeboid’, presumably referring to the hydrostatically driven flow of cytoplasm seen in giant amoebas like Chaos chaos. This nomenclature is unfortunate and ambiguous. Amoebae like Dictyostelium move using actin-based processes as well as blebs, with the ‘mesenchymal’ form predominating in most experiments. Worse, haematopoietic cells such as neutrophils and macrophages are described as ‘amoeboid’ because they have no clearly defined rostrocaudal or dorsoventral axes, unlike for example epithelial cells. However, the amoeboid migration of these cells typically uses a ‘mesenchymal’ actin-based mechanism.

In Dictyostelium the contributions of hydrostatic pressure and blebbing to cell migration are just beginning to be understood. Depending on the osmolarity in which the cells are studied – and therefore the internal hydrostatic pressure – cells will produce either blebs or pseudopods, and can efficiently perform chemotaxis using either [15]. Blebs are also generated upon global cAMP stimulation, and are therefore likely to contribute significantly to the chemotactic response [77]. Thus blebs and pseudopods need not be mutually exclusive, and we frequently observe both in the same cell or even the same protrusion. The definition between the modes of migration may not be sharp in other cell types, and hydrostatic pressure may frequently combine with actin polymerisation.

This information inside the cell to elicit changes in cell morphology and produce motility in the direction of the gradient. Dictyostelium cells, unlike many mammalian cells, are constitutively motile. This means that, in the absence of a chemotactic signal, they randomly produce pseudopods and ‘walk’ around searching for one [16]. When a signal is encountered, cells are able to interpret differences in concentration of as little as 2% over the length of the cell, orientating themselves and moving up the gradient (see [17]). Like most mammalian cells, Dictyostelium cells move by polarising and then stimulating actin protrusions to extend the front of the cell, while keeping the sides free of actin and retracting the rear using a combination of myosin II, other actin-binding proteins, and localised disassembly of actin causing ‘retrograde flow’ from the front to the centre of the cell (Box 1).

Dictyostelium cells detect the gradient of cAMP using heterotrimeric G-protein coupled receptors that activate a number of downstream pathways. The identity of these pathways, and how they interact to induce cell polarity, is therefore key to the understanding of directional control of migration, and as such has been the main preoccupation of a number of groups.
detailed analysis of pseudopod dynamics shows that, in shallow gradients, inhibition of PI3K regulates the rate at which pseudopods are produced, but does not specify nor control their direction [36]. Therefore it is likely that, in the absence of steep gradients, PIP₃ plays a global role, and controls cell-autonomous properties such as the basic rate of cell migration. Indeed, while Dictyostelium cells lacking PI3K remain capable of efficient chemotaxis in strong gradients, both their random motility (in the absence of any gradient) and the speed at which they initially orientate themselves when a gradient is applied are markedly reduced [31,37]. This effect is reproduced in mouse neutrophils, where inhibition of PI3Ky also reduces random motility and the speed at which cells orientate, affecting the absolute number of cells which respond to the gradient but not their speed or directionality once they have responded [38,39].

In steeper chemoattractant gradients, the role of PIP₃ may be different. When PIP₃ is strongly localised towards the signal, it can instruct new pseudopods to form in a defined location. By confining the production of pseudopods to the leading edge, PIP₃ accumulation therefore ensures that, when the signal is strong enough, the cell can move towards the signal with maximum possible speed and efficiency [33].

This changing role of PIP₃ as the external gradient increases is likely to give the best balance of responses in order for a cell initially to find a weak signal in a noisy environment, and then, when the signal becomes clear and strong, to rapidly home in (Figure 1). This behaviour has clear parallels with the problems encountered by cells in the immune system as they track down and close in on their prey, as emphasised by the strong similarities between signalling in Dictyostelium and in neutrophil chemotaxis (Box 1) [2]. It is therefore clear that PIP₃, while not the sole determinant of cell directionality described in a number of papers over the past decade, remains an important component of the chemotactic machinery and contributes to movement towards weak or strong signals [33].

Alternative intracellular signals
The realisation that cells are capable of chemotaxis without PIP₃ has led to a renewed search for other signalling pathways that orientate the cell. One important intracellular pathway induced upon receptor activation involves the conversion of members of the Ras family of small GTPases into their active GTP-bound state [20,40]. The Dictyostelium genome contains an unusually large number of small GTPases, including 15 Ras family members (reviewed in Ref. [41]), although rasG and rasC appear to be the most important for the response to cAMP [42]. Simultaneous loss of both of these genes effectively blocks directional movement, indicating that Ras signalling plays an essential role in chemotactic signalling or movement downstream of receptor activation. In mammalian cells, Ras is known to take part in a large number of essential pathways. The best known of these is PI3K but there are several others that could transduce chemotaxis in the absence of PIP₃.

Another good candidate mediator is the target of rapamycin complex 2 (TORC2). This complex, like PI3K, is activated via Ras upon cAMP stimulation [43], and disruption of different subunits of the complex leads to defects in Dictyostelium chemotaxis [43,44]. TORC2 has also been shown to regulate cytoskeletal organisation in mammalian cells [45,46] where it phosphorylates members of the PKB/Akt family [47]. It was therefore no surprise when a similar pathway was found in Dictyostelium [48]. Interestingly, in this case CAMP stimulation induces the phosphorylation of two distinct PKB isoforms, one of which is recruited to the membrane and phosphorylated in a PI3K-dependent manner, and a second which is constitutively membrane-associated but is locally phosphorylated by TORC2 [48–50]. These different PKB isoforms have as least some functional overlap and can partially compensate for each other [50], indicating that to some extent PI3K and TORC2 work in parallel to mediate similar responses to cAMP. It is important to note that the commonly used PI-3K inhibitor LY294002 also inhibits TOR with a similar Ki, and therefore experiments using this drug should be interpreted with caution [48].

In the search for other PI3K-independent signalling pathways, genetic approaches have been used to screen for Dictyostelium mutants in which chemotaxis is impaired upon treatment with PI3K inhibitors. This identified a member of the phospholipase A₂ family (PLA2) [51] – a result that was confirmed by a second group using cocktails of pharmacological PI3K and PLA2 inhibitors [52]. PLA2 enzymes cleave the second acyl chain of phospholipids, most commonly producing arachidonic acid and lysophospholipids [53] and there is also some evidence that they are important for monocyte migration [54,55].

![Figure 1](image-url) Different roles of PIP₃ in steep and shallow gradients. A diagrammatic representation showing the different localisation of PIP₃ (shown in green) under different conditions. (a) In steep chemoattractant gradients PIP₃ is highly localised to the leading edge, promoting localised pseudopod formation whereas in shallow gradients of chemoattractant (b) there is little, if any enrichment of PIP₃ towards the front. This may reflect the different requirement of the cell to respond when the signal is weak and noisy (c); compared to when it has moved closer to the source and receives a strong, clear signal (d).
The exact roles of PLA2 – its biochemical targets, the proteins it regulates, and the effects on cellular behaviour – remain unclear. PLA2 itself is cytosolic, and does not change its localisation in response to chemoattractant. Indeed, because loss of PLA2 can be compensated for by globally adding arachidonic acid to the medium [51], it seems doubtful that it produces a directional signal at all. Rather, PLA2 may be playing a global role, facilitating the underlying motility of the cell and not its direction. One recent paper [56] strongly supports this idea by finding that PLA2 is important for the type of pseudopods made by cells. Wild type cells usually make pseudopods by splitting previously existing ones [36], making the location of new cells. Wild type cells usually make pseudopods by splitting previously existing ones [36], making the location of new protrusions nonrandom and adding to cell polarization. PLA2 mutants, however, usually generate new pseudopods at random positions [56] so they are less polar; this change may explain their inefficient chemotaxis.

Interestingly, even the loss of both PI3K and PLA2 signalling is insufficient to block Dictyostelium chemotaxis under all conditions. As Dictyostelium cells go through development their behaviour changes; whereas initially they produce and maintain multiple pseudopods and frequently change direction, as they progress through development they become more highly polarised, maintaining a single dominant leading edge. During this constitutively polarised phase, cells lacking both PI3K and PLA2 activity remain capable of chemotaxis despite substantial effects on underlying processes [57].

Under these circumstances, disruption of yet another enzyme, soluble guanylyl cyclase (sGC), is needed to block chemotaxis. sGC appears to have two independent signalling roles, one mediated by the production of cGMP and a second that is cGMP-independent [57]. cGMP itself is unlikely to play the role of a chemical compass as it is too diffusible to produce a directional signal; instead, the requirement of cGMP for the proper assembly of cortical myosin II and the maintenance of cortical rigidity may be the critical factor for PLA2/PI3K-independent chemotaxis [58,59]. This said, sGC is a large protein and also appears to have signalling roles that are independent of guanylyl cyclase activity [57]. Because it binds to actin and localises to the leading edge, sGC may play other as yet unknown roles in directional sensing.

These recent studies, making use of a simple genetic system, have significantly expanded our view of the signalling pathways involved in cell movement, and have made it possible to begin to dissect the relative contributions of each. Importantly, the simple model of a single, linear pathway has been replaced by a more complex world view containing multiple and partially overlapping pathways that govern chemotaxis (Figure 2). This means that genetic experiments must be interpreted with a degree of caution, as the disruption of one pathway can often be circumvented by the compensatory upregulation of another, and while loss of PIP3 signalling has major effects on cell movement, disruption of either PLA2 or guanylyl cyclase alone has little effect unless PIP3 signalling is disrupted as well. It has therefore been proposed that these different signals work as redundant chemical compasses, working semi-independently (and probably feeding back onto one another) to give the cell a robust and flexible system for regulating chemotaxis and cell motility [60,51]. However, we doubt this interpretation, for the reasons discussed below.

**Integration of signals and cytoskeleton: can chemotaxis be explained using linear pathways?**

Dictyostelium has been a successful and productive tool for experiments in chemotaxis. Researchers throughout the world have identified a number of genes and associated pathways that are important for efficient chemotaxis (Figure 2). Nevertheless, many of the pathways discussed above do not seem to generate signals that are spatially localised in the manner expected for compass-based models. It may be that by disrupting them we are merely interfering with normal cell movement, rather than breaking the compass. It is therefore important to ask if we even need a strong internally localised signal (or signals) to steer the cell.

It was recently shown that both Dictyostelium and mammalian cells rarely make de novo pseudopods – new pseudopods in locations where none previously existed – even when turning. Rather, they make new extensions by...
different chemoattractants, adhesion receptors, and intra-accelerates, stabilises, or disrupts pseudopod dynamics to feedback loops.

This type of system would allow many factors including inhibition) mechanisms [67]. This suggests an appealing role might be seen for cGMP which, by stimulating myosin II assembly, is able to stabilise cell polarity and maintain a leading edge in whichever orientation it forms [58]. Whereas the connection between cGMP and myosin II is specific to Dictyostelium, in mammalian cells it is likely that a similar function can be assigned to the small GTPase Rho and its associated kinase, ROCK, that are absent from the Dictyostelium genome but induce contractility in a similar way, and could thus coordinate the behaviour at the front and back of the cell.

The important question, therefore, concerns how these different pathways impinge on the cytoskeletal organisation and how they are integrated. This is a complicated problem and one that cannot be described by a series of linear pathways. Progress will require a combination of approaches such as systems biology and pathway modelling, but above all a more complex world view that considers interacting pathways rather than single proteins or genes.

Prospects and unanswered questions

Our picture of the processes driving cell migration has rapidly evolved over the past few years, but a number of major questions remain. Primarily, we need to know how the multiple signalling pathways involved in directing the cell are integrated, and to clarify which of these actually change the directionality of the cell, and which simply alter its general underlying motility. This has started to be done with the TORC2 pathway where a number of PKBR1 targets have been identified [48]. These include adhesion molecules such as talin, but also include some RasGEFs and a RhoGAP – which presumably have their own pleiotropic effects that overlap with other pathways. The physiological roles of both PLA2 and PKBA however remain elusive; more work is required to clarify these.

Descriptions of chemotaxis that depend on biasing random movement are not understood at a mechanistic level. It appears that cells move by comparing multiple pseudopods and by choosing which one to maintain, but we do not understand how the comparison is achieved. Observations indicate that relative receptor occupancy at each pseudopod is crucial [36], but it is unclear how the relative levels are compared and how this is converted into cytoskeletal activity. The earlier emphasis on the induction of new pseudopods by spatially determined signals in steep gradients has hindered the development of models that involve slight bias. Likewise, the concept of actin waves also requires more study – as yet we do not clearly understand what causes them, nor their physiological relevance. Waveform signalling events are inherently complex and do not easily fit into linear signalling frameworks, but this will have to be resolved if we are to understand the basic mechanisms regulating the cytoskeleton. It is possible that a more subtle application of the genetics of Dictyostelium...
will help us to do this. Perhaps wave behaviours will also provide a framework for understanding how random motility is biased by small signals combining two subfields.

There are also several other results that remain unexplained. For example, functional NSF (N-ethylmaleimide sensitive factor) and therefore membrane trafficking is essential for cell movement, yet the exact role of such trafficking is unclear [68,69]. In addition, clathrin was also shown unexpectedly to contribute to the maintenance of cell polarity by an undetermined mechanism [70], indicating that the endocytic pathway plays a role. There is evidence that membrane tension limits the extent of normal protrusions [71] and it is possible that *Dictyostelium* will provide a good target for studying how such basic cell biological processes are involved in cell polarity and movement.

**Conclusion**

In this review we have outlined the ways in which *Dictyostelium* can be used to study cell migration. Although some of the intricacies of intracellular signalling in mammalian tissue cells are absent, *Dictyostelium* provides a relatively simple experimental target in which to understand fundamental principles of cell migration that can be generally extrapolated.

Recently there has been considerable change in our understanding of both the underlying principles of directed migration and of the signalling pathways involved. In particular, our understanding of PI(3,4,5)P3 signalling and the important roles it plays in ensuring efficient migration has rapidly evolved, as has the need to search for alternative interconnected pathways and theoretical frameworks to describe complex chemotactic behaviour.

As always, it is important to ask whether we are doing the best and most appropriate experiments to answer more general questions. In the case of cell motility these questions are often difficult to answer in other cell types, and discoveries in *Dictyostelium* have frequently proven to be generally applicable. Obviously, *Dictyostelium* cannot accurately represent the complex situation and unique problems of migrating through a tissue, but while we still struggle to understand the basic principles of how a cell is able to respond to chemotactic cues and regulate its shape, the gregarious social amoeba will remain enormously useful.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tcb.2009.07.004.

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