Chemotaxis of a model organism: progress with *Dictyostelium*

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Abstract

Model organisms have been key to understanding many core biological processes. *Dictyostelium* amoebae have the attributes required to perform this role for chemotaxis, and by providing an evolutionary distant reference point to mammalian cells, they allow the central features of chemotaxis to be discerned. Here we highlight progress with *Dictyostelium* in understanding: pseudopod and bleb driven movement; the role of the actin cytoskeleton; chemotactic signal processing, including how cells adapt to background stimulation, and the controversial role of PIP3. Macropinocytosis and the axenic mutations are raised as potential confounding factors, while the identification of new players through proteomics holds great promise.

Introduction

Amoeboid cells migrate using their actin-myosin cytoskeleton to produce extensions of their plasma membrane and movement of the cell body. This style of movement probably dates back to the phagotrophic ancestor of metazoa and amoebae, and today much of our understanding of chemotaxis comes from studies on two descendants of this ancient organism: *Dictyostelium* amoebae and neutrophils or similar mammalian cells [1,2]. Both are fast moving, acutely chemotactic cells, which also use their cytoskeleton for large-scale ingestion by phagocytosis and macropinocytosis. *Dictyostelium* amoebae do not need chemoattractant gradients to move, but use chemotaxis to folate to hunt bacteria during growth, and chemotaxis to cyclic-AMP to attract each other into multicellular aggregates following starvation. In what follows we cover progress over the last two to three years in understanding *Dictyostelium* chemotaxis (Figure 1).

Modes and mechanics of movement

Dictyostelium cells adapt how they move to their physical environment. On smooth, planar coverslips they move mainly with F-actin-driven pseudopods, but when they face mechanical resistance, such as an elastic overlay, they move with blebs [3] – extensions of the plasma membrane formed when it detaches from the underlying cortex and is driven out by fluid pressure. Cells can even swim if the opportunity arises [4]. On a surface, cells switch smoothly from pseudopods to blebs as the mechanical resistance increases and these cooperate at the leading edge by triggering each other's formation: pseudopods can trigger blebs on their flanks by producing areas of negative membrane curvature [5], and blebs can produce pseudopods by continued actin polymerization as they restore their cortex [3]. Both blebs and pseudopods can be orientated by chemotactic gradients and cells forced to make an acute turn often also produce actin micro-spikes up-gradient [3]. It thus appears that chemotactic signalling can target three different structures - pseudopods, blebs and micro-spikes. Blebbing increases as cells develop [3], perhaps in preparation for multicellular development, where extensive morphogenetic movements shape the eventual stalked fruiting body.

The seemingly endless variety of cell shape seen in chemotaxis can be reasonably well described mathematically by just three principal components, representing cell elongation, cell polarisation and pseudopod splitting or bending [6]. Cells adopt characteristic shapes according to the strength (signal/noise) of the chemotactic gradient: in strong gradients they tend to be wedge shaped and move quickly, whereas in weak gradients they typically alternate between pseudopod splitting and cell elongation.

Very little of the force that cells exert on their substratum is needed to overcome inertia or friction, instead most is due to balanced contractile forces linked through the plasma membrane to cell adhesions. As well as in-plane forces towards the centre, cells also pull up and push down on the substratum [7,8]; the main effect of the forces within the cell may be to produce fluid pressure, driving cytoplasmic flow and allowing cells to make blebs when required.

The actin cytoskeleton

Movement is powered by the actin cytoskeleton, which is organised around the cell cortex, and is the ultimate destination of chemotactic signalling. Actin polymerisation in pseudopods is triggered by the SCAR/WAVE complex acting through the Arp2/3 complex. Arpin, a recently discovered inhibitor of Arp2/3 opposes SCAR, and when deleted results in fast-moving cells [9]. Somewhat surprisingly, cells can do without SCAR, moving mainly with blebs when SCAR is deleted and replacing it with WASP in the remaining pseudopods [10,11]. SCAR activation probably involves the loss of inhibitory phosphorylations at the C-terminus, as well as activation from Rho-family small GTPases. However, the signalling route from chemoattractant receptor to actin polymerisation remains unclear: for instance systematic knock-out of RhoGEFs with Dbl-domains has not so far yielded a clear candidate [12] whereas some mutants of the Dock/Zizimin family of GEFs do show clear defects in movement or actin polymerisation [13,14].

The actin cytoskeleton is poised for excitability [15]. One of the most enigmatic phenomena related to cell motility is the actin waves visualised on the basal surface of cells by TIRF microscopy. These are not so much waves as patches in which a core of PIP3 is surrounded by a zone of actin polymerisation on all sides. These patches can expand or contract and move around; when patches collide with the edge of the cell they can cause some cell movement, but their organization suggests they are not the precursors of pseudopods, but are best viewed as frustrated phagocytic or macropinocytic cups [16,17]. Cells also produce spontaneous, regular foci of actin polymerisation on their basal surface, with a period of around 10 seconds and independently of chemotactic stimulation, suggesting the existence of local positive feedback loops driving actin polymerisation [18].

When cells are acutely stimulated with chemoattractant there is a rapid and transient polymerisation of actin and the recruitment of it, and many other relevant proteins, into a crude detergent-resistant, cytoskeletal fraction [19]. In a pioneering approach these recruited proteins have been catalogued by mass-

spectroscopy using SILAC heavy isotope labelling to provide an internal standard from unstimulated cells [20]. The Arp2/3 complex is the most abundant actin nucleator recruited, and the Cap32/34 complex the most abundant capping protein; among RacGEFs, DockA and DockB are recruited first with the Dock-interacting protein, ElmoE, also strongly recruited.

A wide array of cytoskeletal reporters are available [21] to which can be added a reporter for active Rac [22]. A detailed comparison of F-actin reporters showed that they can recognize different portions of the cytoskeleton, such as the cortex, and that LifeAct is the most representative reporter of F-actin in general [23].

Receptor-proximal signalling and adaptation

Cyclic-AMP, the most studied chemoattractant, is detected by a G-protein-coupled receptor, cAR1, and its attendant G-protein, which are both genetically essential for chemotaxis. Many cellular responses to cyclic-AMP are transient, and adapt (die down) at constant cyclic-AMP levels [24]. Adaptation of some form lies at the heart of chemotactic signalling. It allows cells to orientate in gradients spanning a wide range of chemoattractant concentration and provides them with a memory of their prior exposure to chemoattractant. Adaptation can be simply explained by two opposing processes triggered from the receptor: excitation and delayed inhibition. If the adaptive processes spread to different degrees within the cell – with excitation being more local – then they can allow cells to read chemotactic gradients and bias protrusions up-gradient [25]. Consistent with this sort of scheme, local stimulation of the membrane with cyclic-AMP tethered to a bead causes only local production of PIP3, which does not spread to encompass the rest of the membrane [26].

Adaptation is also manifest in the back of the wave problem. Why do cells not reverse their direction in the back of natural waves of cyclic-AMP as they sweep over them during aggregation? This situation has been reproduced using flow chambers to create travelling waves of cyclic-AMP, and provided their period approximates the 5 minute period of natural waves, cells do not reverse in these waves either [27,28]. The underlying mechanism for this behaviour awaits discovery.

Although the output of an adaptive module adapts, the excitatory and inhibitory processes within it are not expected to – they must continuously report the presence of chemoattractant. Neither G-protein activation [29], nor receptor numbers adapt in the short term, consistent with them producing a continuous activating signal so long as cyclic-AMP is present. Similarly, it was shown more than 20 years ago that cAR1 becomes phosphorylated on its C-terminal tail with slower kinetics than receptor activation and that this phosphorylation also does not adapt [30], suggesting that it might be the seat of adaptation. However, these initial studies found that a non-phosphorylated receptor could support development and apparently normal chemotaxis and cyclic-AMP relay, arguing against a crucial role in adaptation [31]. Recent work suggests a revision of this view: using improved assays, cells with non-phosphorylatable cAR1 receptors

can be seen to chemotax poorly and produce cyclic-AMP for prolonged periods, indicating that at least some adaptive processes emanate from receptor phosphorylation [32].

TORC2 is activated transiently by both cyclic-AMP and folate, but does not cross-adapt to them [33], and satisfyingly, a similar conclusion was reached for Ras activity monitored in single cells [29]. This suggests that adaptation in this pathway occurs above the level of Ras activation, possibly close to the receptor.

Phosphorylated G-protein coupled receptors provide binding sites for arrestins giving a platform for signalling beyond the classically described receptor down-regulation. *Dictyostelium* potentially has six arrestins, with the partially redundant pair, AdcB and AdcC, involved in cyclic-AMP signalling [34]. An arrestin reporter (AdcC-mCherry) is recruited to the plasma membrane independently of Gβ when cells are stimulated with cyclic-AMP, and as cAR1 becomes phosphorylated. Recruitment depends at least partially on the C-terminal tail of cAR1 but is transient, unlike tail phosphorylation, showing that other factors must be involved. Spontaneous cyclic-AMP signalling is faster in an *adcB-/adcC-* double mutant, suggesting that arrestins are involved in setting the frequency of cyclic-AMP waves. Somewhat surprisingly, no chemotactic defect was reported, although this was not investigated in detail.

Two further proteins have been identified which operate high in the signal transduction pathway. Ric8 binds the Gα subunit of heterotrimeric G-proteins, acting as a GEF, and is suggested to prolong the life of the GTP form of Gα after receptor stimulation, making it important for chemotaxis in weak gradients [35]. Elmo and Dock proteins form complexes which act as GEFs for Rac-type small GTPases. ElmoE potentially forms a direct link from the heterotrimeric G-protein to RacB and actin polymerization, as it physically associates with Gβ, RacB and F-actin. Null mutants have a mild chemotactic phenotype and a defect in actin polymerization stimulated by cyclic-AMP [36].

Phosphoinositide and Ras signalling

The *Dictyostelium* phosphoinositides are now known to be chemically unusual, using an ether linkage in their lipid backbone, and are thus plasmanylinositides rather than phosphatidylinositides [37]; however this feature is not known to make any difference to recognition by cytoplasmic proteins such as those carrying PH-domains. PIP3 first came to prominence in chemotaxis with the finding that latrunculin-treated cells formed arcs of PIP3 towards a needle releasing cyclic-AMP [38]. Once thought of as a compass guiding movement, the significance of this phenomenon is now much less certain, as PIP3 gradients are genetically dispensable for chemotaxis to both cyclic-AMP and folate [39,40].

The most common manifestation of PIP3 is as intense patches in the plasma membrane. These patches must be maintained despite the rapid diffusion of PIP3 in the membrane and result from excitable kinetics of PIP3 production [41]. Although PIP3 patches can be orientated by chemotactic gradients, most frequently they template macropinosomes – large endocytic structures – that

largely depend on PIP3 for their formation [40]. PIP3 effectors are recruited to the membrane by binding to PIP3 and include the protein kinase, PKB, and a number of class I myosins [42]. A triple knockout of myoID/E/F produces chemotaxis and actin polymerisation defects, which surprisingly, are stronger than those of a PI3-kinase quintuple mutant, suggesting that the myosins can function independently of PIP3.

PIP2, the substrate for PI3-kinase and phospholipase-C, and an anchor for many proteins to the plasma membrane lies at a pivotal point in signalling. Direct measurement suggest that PIP2 is not globally depleted by chemotactic stimulation of cells [37] although local depletion can occur in PIP3 patches [17]. One reason why PIP2 levels are sustained during signalling is that the PI4P5-kinase, PikI, is activated by chemoattractant-driven phosphorylation. In the absence of PikI, PIP2 levels are severely depleted and chemotactic signalling is interrupted after the activation of Ras [43].

Ras is activated from cAR1 independently of Gα2 [44] and the pair of Ras genes RasC/G had been accepted as central to chemotactic signalling [1,2]. Their importance is less clear after reports that a RasG-/C- double mutant can chemotax with near wild-type efficiency to folate [39]. A follow-up investigation showed that the discrepancy with earlier work is largely due to the measurement conditions, but confirmed that some chemotaxis is possible in the absence of RasG and RasC [45] indicating the existence of a signalling pathway independent of these two proteins. The broad defects of mutants in Daydreamer, a Ras effector, further hint that Ras has a wide reach and its role is not restricted to, for instance, direction sensing [46].

Most laboratory work on *Dictyostelium* uses axenic mutants that can grow in liquid medium due to increased rates of macropinocytosis. The key locus responsible, *axeB*, has now been identified as encoding the RasGAP, NF1 [47], which is deleted in standard axenic strains, such as Ax2 and Ax3. In growing cells, the resultant activation of Ras and PI3-kinase and excessive formation of macropinosomes interferes with folate chemotaxis, which, ironically, can then be improved by deleting PI3-kinase [48]. Macropinocytosis declines as cells starve, but the remaining macropinosomes can easily be mistaken for pseudopods in confocal sections, potentially confounding studies on cyclic-AMP chemotaxis.

Summary and Prospects

Several key advances have been made in recent years that hold promise for the future, perhaps most importantly the increased knowledge of receptor proximal events and adaptation. The biological context of chemotaxis is also better understood, with the ability of cells to switch between pseudopod and bleb modes of movement paralleling work with mammalian cells and raising questions of how this switch is regulated. PIP3 signalling is also being clarified, with its most obvious genetic role being in macropinocytosis. The almost universal use of axenic cells, in which Ras/PIP3 signalling and macropinocytosis are upregulated is a serious confounding issue, which the use of wild-type cells should help to resolve.

Great use has been made of modelling (not specifically covered here, for which we apologise), principally conceptual modelling to help make ideas explicit. Ultimately it will be essential to progress to realistic models, but this is not possible yet because the components and pathways mediating chemotaxis are incompletely known. Genetic screens are a powerful route to understanding these pathways, but struggle where there is redundancy, as seems to be the case with chemotaxis. Therefore other routes are needed, and SILAC mass spectroscopy works well with *Dictyostelium* and has already proven its value in identifying proteins recruited to the cytoskeletal fraction following chemotactic stimulation. It can also be used to examine protein phosphorylation on a proteome-wide scale [49], which promises to be a powerful approach.

Finally, chemotaxis to cyclic-AMP is embedded in a particular developmental context, and cyclic-AMP causes many cellular responses, such as transcriptional pulsing, that may be more related to development [50,51] than to chemotaxis. Therefore it is extremely valuable to examine chemotaxis to folate as well as to cyclic-AMP, so that specific and universal attributes can be discerned.

Acknowledgements

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Figure legend

Figure 1. *Dictyostelium* chemotaxis; signal transduction and related membrane projections.

(a) Cyclic-AMP binding to the cAR1 receptor initiates dissociation of heterotrimeric G-proteins to trigger downstream signalling. Gβγ subunits can associate with ElmoE, which in turn associates with Dock-like proteins (DocC and ZiziminA) to regulate RacB-mediated actin polymerization. Gα activity is regulated by the non-receptor GEF, Ric8. Sustained stimulation leads to receptor phosphorylation (by an unidentified GRK) and association of an arrestin, AdcC. These in turn regulate G-protein independent signalling pathways involved in adaptation.

(b) Both the actin cytoskeleton and Ras/PI3K activity are excitable and influenced by stochastic noise. Inputs from chemoattractant receptors feed into these excitable modules to trigger signalling events and formation of actin structures. Incoherent feed-forward pathways and other mechanisms of adaptation influence Ras/PI3K signalling. Previous states of the signalling network can influence the current state via cellular memory.

Dictyostelium cells can form a range of projections of their plasma membrane. Microspikes emerging from the actin cortex are observed up-gradient when cells reorientate, and often precede blebbing. Blebbing is most frequent during chemotaxis in resistive environments and is positively regulated by PIP3, PIP3-binding proteins and myosin. In the absence of SCAR/WAVE, the frequency of blebs is greatly increased. Pseudopods are the primary mode of amoeboid chemotaxis. As actin-driven structures, they are positively regulated by Rac and SCAR/WAVE, with Arpin proposed to restrict actin polymerization during chemotaxis. Macropinosomes are abundant in axenic strains due to the loss of the RasGAP, NF1, and form from patches of intense Ras/PIP3 signalling. They are used for feeding by fluid uptake, not for chemotaxis, but can easily be mistaken for pseudopods in confocal sections.

Figure 1

(a) Receptor proximal events

(b) Signalling and the cytoskeleton

(c)

	Microspikes	Blebs	Pseudopods	Macropinosomes
Conditions	Reorientation to new gradients	High resistance environments	Wild type chemotaxis	Axenic growth
Molecules	?	PIP3, PhdA CRAC, Myosin SCAR/WAVE	Rac, SCAR/WAVE Arpin	Ras, PIP3 SCAR/WAVE NF1
Cellular response				