

Chemotaxis: insights from the extending pseudopod

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Summary

Chemotaxis is one of the most fascinating processes in cell biology. Shallow gradients of chemoattractant direct the movement of cells, and an intricate network of signalling pathways somehow instructs the movement apparatus to induce pseudopods in the direction of these gradients. Exciting new experiments have approached chemotaxis from the perspective of the extending pseudopod. These recent studies have revealed that, in the absence of external cues, cells use endogenous signals for the highly ordered extension of pseudopods, which appear mainly as alternating right and left splits. In addition, chemoattractants activate other signalling molecules that induce a positional bias of this basal system, such that the extending pseudopods are oriented towards the gradient. In this Commentary, I review the findings of these recent experiments, which together provide a new view of cell movement and chemotaxis.

Key words: *Dictyostelium*, Actin, Cell movement, Chemotaxis, Pseudopod

Introduction

Many cells have a mode of migration known as amoeboid movement that is characterised by frequent changes in cell shape as a result of the extension of protrusions (Friedl and Wolf, 2009; Lammermann and Sixt, 2009). The protrusions of an amoeboid cell are often termed pseudopods or lamellipods; these protrusions can adopt different shapes that are referred to as thin filopodia or bulbous lobopodia. Pseudopods are crucial for cell movement, because they determine the speed, direction and trajectory of the cell. Adjacent cells can coordinate pseudopod extensions, thereby contributing to collective cell migration in addition to other processes such as contact guidance (Weijer, 2009). An important aspect of cell motility is the ability of cells to respond to directional cues with oriented movement. Gradients of diffuse chemicals give rise to chemotaxis (Hoeller and Kay, 2007; Weiner, 2002). Other directional cues that can induce oriented movement are temperature gradients (thermotaxis) or electric fields (electrotaxis) (Bahat and Eisenbach, 2006; Zhao, 2009), which will not be covered here. These signals somehow modulate the direction of pseudopods such that, on average, cells move in the direction of the positional cues.

How do amoeboid cells move and navigate using chemical gradients? Many experiments address this question using a strategy that involves exposure of cells to a gradient of chemoattractant, followed by measurement of the spatiotemporal activation of signalling molecules that ultimately enable oriented movement. This 'signal-centred' approach to understanding cell movement is a very powerful means by which the signalling pathways involved can be identified, and the fundamental mechanisms that underlie gradient sensing, symmetry breaking (which can be involved in determining cell polarity or the formation of a leading edge) and signal amplification (which generates an intracellular gradient of signalling molecules that is much steeper than the extracellular chemoattractant gradient) elucidated (Franca-Koh et al., 2006; Insall, 2010; King and Insall, 2009; Merlot and Firtel, 2003; Schneider and Haugh, 2006). Many of these experiments have been carried out with *Dictyostelium*. This genetically tractable organism moves in a similar manner to many other amoeboid cells and exhibits chemotaxis in response to very shallow gradients of the extracellular chemoattractant cyclic AMP (cAMP) (Kay et al.,

2008). A shallow gradient of cAMP induces the activation of cAMP receptors and their associated G-proteins in a manner that is approximately proportional to the steepness of the gradient and is only slightly stronger at the leading edge than at the rear of the cell. By contrast, the small GTPase Ras and many downstream components are activated much more strongly at the leading edge than at the rear of the cell (Jin et al., 2000; Zhang et al., 2008). At least four signalling pathways contribute to chemotaxis of *Dictyostelium* cells: the phosphoinositide 3-kinase (PI3K) pathway that produces phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P₃], which in turn activates the Akt (also known as PKB) pathway; the TorC2 pathway, which activates PKBR1, a soluble guanylyl cyclase (sGC) that is activated at the leading edge and produces cGMP; and a pathway involving PLA2, which has an unknown mechanism (Kamimura et al., 2008; Veltman et al., 2008). Owing to the multitude of signalling pathways and their complex regulation by positive and negative-feedback loops, it is difficult to establish a connection between the signalling pathways and the locomotion apparatus in *Dictyostelium* and other organisms, and to identify how cells actually extend pseudopods in the direction of the gradient.

Recently, investigators have applied a complementary 'pseudopod-centred' approach to gain understanding of cell movement. In this approach, investigators observe in detail how cells extend pseudopods, then try to integrate these observations with what is known about established signalling pathways (Andrew and Insall, 2007; Arrieumerlou and Meyer, 2005; Bosgraaf and Van Haastert, 2009a; Insall, 2010; Li et al., 2008; Maeda et al., 2008; Takagi et al., 2008). It has been suggested that pseudopods are self-organising structures, which means that their organisation is largely intrinsically controlled (Karsenti, 2008). Although external signals can trigger the formation and location of a pseudopod, the pseudopod otherwise follows a typical life cycle (see Box 1). The recent focus on pseudopods follows up pioneering work that was carried out in the 1980s that involved computer-assisted analysis of cell movement, pseudopod extension and chemotaxis (Potel and Mackay, 1979; Varnum-Finney et al., 1987; Varnum and Soll, 1984). Modern live-cell imaging and improved computer algorithms now allow more refined cell and pseudopod analysis. In studies that take the

Box 1. Three fundamental characteristics of pseudopods

Pseudopods are self-organising structures (Andrew and Insall, 2007; Bosgraaf and Van Haastert, 2009a; Karsenti, 2008)

External signals can initiate the formation of a pseudopod, but the pseudopod then undergoes a series of shape changes independently of external signals. This implies that chemoattractants can modulate the time and position at the cell surface where the pseudopod will form, but otherwise will have limited effects on other properties, such as growth period or length of the pseudopod.

Pseudopods are extended perpendicular to the cell surface (Mogilner and Oster, 1996; Van Haastert and Bosgraaf, 2009a)

The direction of the extending pseudopod depends on the position where the pseudopod starts, in combination with the curvature of the surface at that position of the cell. This implies that, during navigation in chemotactic gradients, pseudopods do not bend in the direction of the gradient. For directional movement, pseudopods must form at the side of the cell closest to the gradient. A cell with a very irregular shape cannot chemotax properly.

Pseudopods are formed de novo or by splitting of the current pseudopod (Andrew and Insall, 2007; Bosgraaf and van Haastert, 2009b)

A pseudopod can start within the region of an existing pseudopod (splitting) or from the cell body (de novo). Splitting pseudopods are extended preferentially, alternating right or left at small angles, causing the cell to take a persistent zigzag trajectory. De novo pseudopods are extended in a random direction. This implies that the ratio of splitting pseudopods to de novo pseudopods determines the persistence of cell movement. In terms of chemotaxis, persistence of cell movement functions as a memory and integrator of directional information. Mutant cells that have defects in pseudopod splitting have very poor persistence, do not effectively move through the environment and have impaired chemotaxis.

pseudopod-centred approach, large data sets are collected on the spatiotemporal properties of pseudopods that are extended by cells in the absence or presence of directional cues. In addition, studies of cells carrying mutations in genes that encode the various proteins involved in cell movement and chemotaxis are beginning to uncover the mechanisms by which specific signalling pathways induce pseudopod formation in the direction of a chemoattractant gradient. In this Commentary, I summarise these recent experiments and discuss the implications of these collective findings for the cell biology of pseudopod extension, cell movement and chemotaxis.

Pseudopod measurements

Traditionally, cell movement is measured by following the position of the centroid of the cell in time, which is defined as the geometric centre of mass or perimeter of the two-dimensional image of the cell. Simple computer algorithms can detect this centroid and dissect the trajectories into discrete steps and turns (Li et al., 2008; Soll et al., 2003). However, although centroid tracking has provided important details about amoeboid movement (Li et al., 2008; Shenderov and Sheetz, 1997), it cannot be used for quantitative analysis of pseudopods, because only a small fraction of a cell's pseudopods cause a change in its direction of movement (Bosgraaf and van Haastert, 2009b). Therefore, other algorithms are used to identify the outline of the cell and to define a pseudopod on the

basis of an extending convex area (Machacek and Danuser, 2006; Soll et al., 2003). A simple and attractive description of a pseudopod is a vector that connects two points on the cell surface: where the protrusion started and stopped growing, respectively (Bosgraaf and Van Haastert, 2009c). Measurements of vectors representing thousands of pseudopods can be used for statistical analysis of pseudopod properties – including frequency, size, growing time and the direction of movement towards chemoattractants. Furthermore, analysis of these vectors with autocorrelation algorithms can provide information about long-term interactions of pseudopods (see below), an understanding of which has been instrumental in uncovering the stochastic order of pseudopod formation in the absence of external cues (Bosgraaf and van Haastert, 2009b).

Pseudopod splitting and de novo pseudopod extension

By observing the position on the cell surface where pseudopods are extended, Andrew and Insall discovered that pseudopods are frequently formed by splitting of an existing pseudopod. Two forms of splitting have been observed in *Dictyostelium* and other organisms (Andrew and Insall, 2007; Bosgraaf and van Haastert, 2009b). In a 'Y-split', an organelle-free protrusion splits into two pseudopods, both of which grow in size, and then usually one pseudopod is retracted while the other is maintained. In a 'one-way' split, a new pseudopod is formed at the side of the current pseudopod; the new pseudopod grows for some time, stops and then a new pseudopod is formed at its side. In polarised cells, one-way splits dominate over Y-splits, probably because the formation of one-way splits better retains cell polarity. In addition to the formation of a new pseudopod by splitting of an existing pseudopod, sometimes a pseudopod is formed de novo, i.e. on a part of the cell surface that has not been involved in pseudopod extension for some time. As will be argued below, pseudopod splitting forms the basis for persistent cell movement and navigation. Fig. 1 shows a *Dictyostelium* cell that forms a de novo pseudopod, which is followed by three one-way splits.

Pseudopods are extended perpendicular to the cell surface

An extending protrusion generates counter forces in the plasma membrane and, energetically, the most favourable direction of the pseudopod is perpendicular to the membrane (Mogilner and Oster, 1996) (Fig. 2). This hypothesis was tested for pseudopods extended by *Dictyostelium* cells in buffer and by cells navigating in a gradient of chemoattractant. Indeed, morphometric analysis showed that cells always extend pseudopods perpendicular to the cell surface with a relatively small standard deviation (Van Haastert and Bosgraaf, 2009a). The gradient of chemoattractant does not induce bending of this pseudopod (statistically by less than 2 degrees). To move in the direction of a gradient, a cell must form many pseudopods at the side of the cell that is facing the gradient. In addition to this positional bias, efficient chemotaxis requires that the cell has a smooth ellipsoid shape (Fig. 2). This was demonstrated in studies of a *Dictyostelium* mutant that has an irregular shape owing to the deletion of formin; although pseudopods originated at the front of the cell, they were found to extend in many different directions, resulting in poor chemotaxis (Van Haastert and Bosgraaf, 2009a). The notion that pseudopods are always perpendicular to the cell curvature implies that the direction a pseudopod follows is determined by the position on

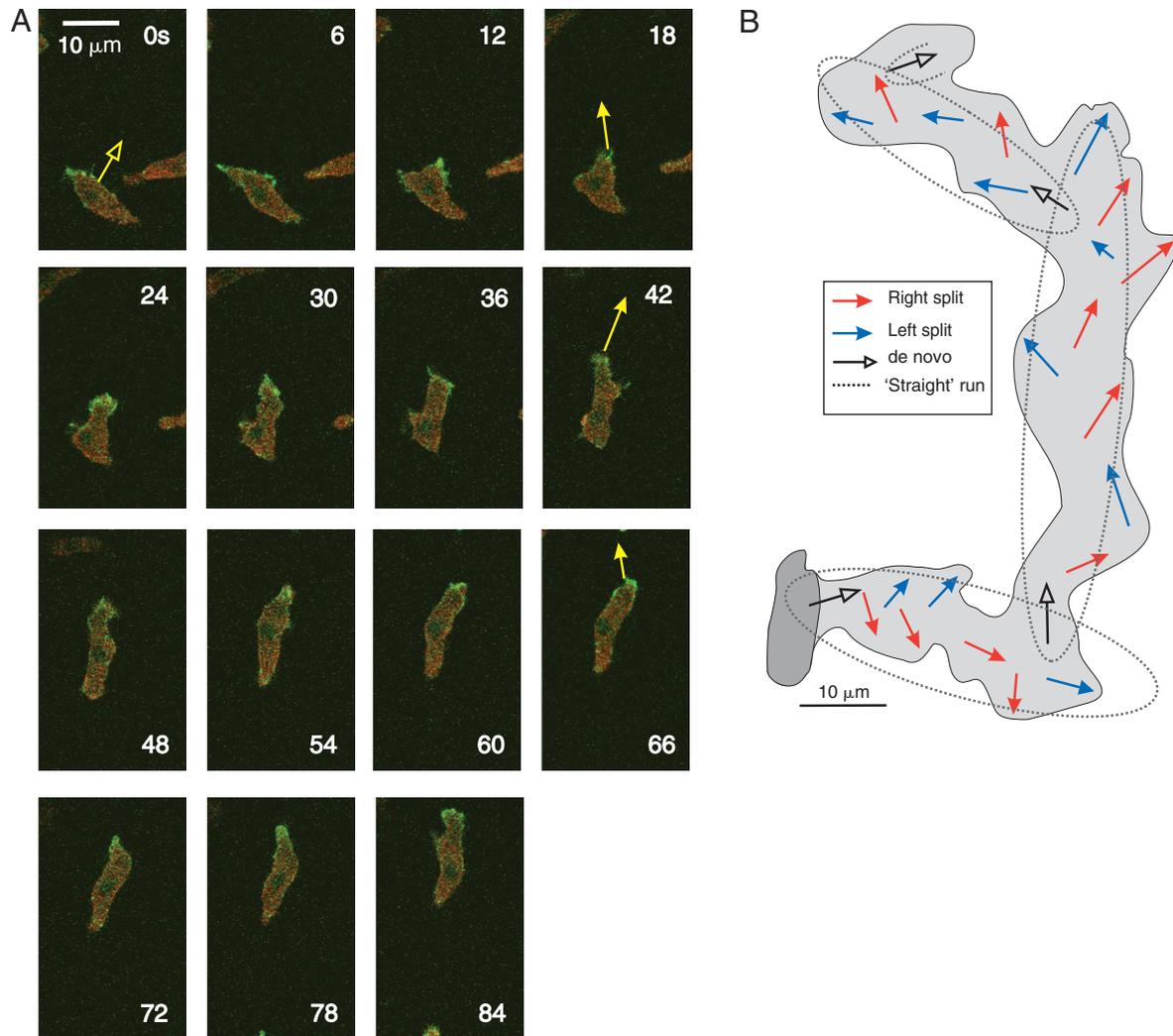


Fig. 1. A *Dictyostelium* cell moving in buffer. (A) Pseudopods are indicated by yellow arrows just before a pseudopod is formed, and are either de novo (0 seconds; indicated by empty arrowhead) or formed by splitting of the current pseudopod (18, 42, 66 seconds; indicated by filled arrowheads). (B) A 15 minute track of the cell. The dark grey area indicates the cell at the start, whereas the light grey area represents the surface covered by the cell during movement. The track illustrates the outcome of statistical analysis of a few hundred pseudopods: splitting occurs preferentially alternating right–left at a small angle, which causes the cell to continue moving in the same direction. Conversely, a de novo pseudopod can be extended in any direction, thereby inducing a random turn.

the cell surface where the pseudopod is formed, in combination with the curvature at that position.

Pseudopods extend in an orderly fashion in the absence of external cues

Work in the previous century has demonstrated that amoeboid cells exhibit a so-called correlated random walk: the direction of a cell's current movement is correlated with that of its movement in the past, and cells therefore move with persistence (Gail and Boone, 1970; Patlak, 1953; Potel and Mackay, 1979). The duration of this correlation is the persistence time, which for many amoeboid cells in buffer is a few minutes, during which time around ten pseudopods are extended. Statistical analysis of pseudopod splitting and de novo pseudopod formation in *Dictyostelium* revealed that the angle between two splitting pseudopods is ~ 55 degrees, and that a split to the right is frequently followed by a split to the left (Bosgraaf and van Haastert, 2009b). This alternating splitting at a relatively small angle leads to a persistent zigzag trajectory. By

contrast, de novo pseudopods are extended in any direction, have no right–left preference relative to the previous or next pseudopod, and therefore induce a random turn in the direction of a cell's movement. As a consequence, the persistence time of movement depends on the ratio of splitting and the formation of de novo pseudopods.

Studies of *Dictyostelium* mutants revealed that de novo pseudopod formation is inhibited by a cGMP pathway that induces myosin filaments in the cortex of the cell body, whereas pseudopod splitting is facilitated by PLA2 signalling through an unknown mechanism (Bosgraaf and van Haastert, 2009b). These signalling pathways are used by cells to modulate food-searching behaviour (Van Haastert and Bosgraaf, 2009b). Cells with ample food have low PLA2 and cGMP activity, and form many de novo pseudopods with random turns, causing brownian-like motion in a small area. Starvation induces PLA2 and cGMP activity, thereby strongly enhancing pseudopod splitting, leading to persistent cell movement. As a consequence of enhanced splitting, starved cells visit a much

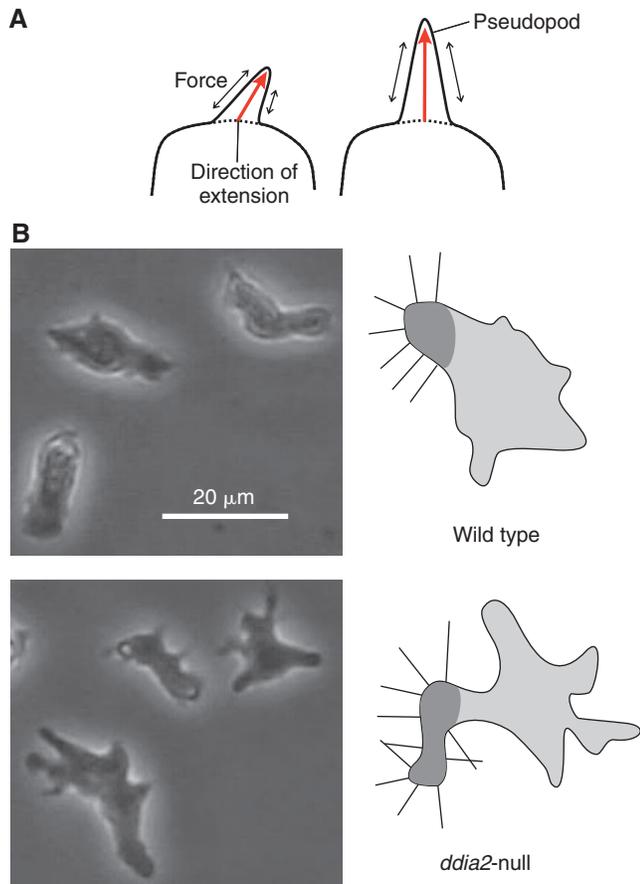


Fig. 2. Pseudopods are extended perpendicular to the cell surface. (A) Unequal forces at the membrane surrounding a protrusion that is not perpendicular (left) drive the pseudopod perpendicular to the cell surface (right). (B) The pseudopods that are induced at the side of wild-type cells that are facing the gradient (dark-shaded area in schematic on right) are directed well towards the gradient. *ddia2*-null cells, which have a very irregular surface curvature, show impaired chemotaxis, even though pseudopods also form at the side of the cell that faces the gradient.

larger area per unit of time, although they do not extend more pseudopods than non-starved cells. It appears that the basis for persistent cell movement is the balance of alternating right-left pseudopod splitting and random turns by de novo pseudopods. In addition to its role in food searching, this persistent pseudopod extension is exploited for efficient navigation in chemoattractant gradients.

From gradient to pseudopod extension during chemotaxis

Experiments with chemoattractant-filled micropipettes have revealed that exposed cells rapidly extend pseudopods in the direction of the gradient (Gerisch and Keller, 1981). Such experiments give the impression that the gradient induces the pseudopod – i.e. the cell reads the gradient – and then, based on the upgradient accumulation of signalling molecules, extends a pseudopod on that side (Bourne and Weiner, 2002; Meili and Firtel, 2003; Weiner, 2002). From this perspective, it has been postulated that the small concentration difference in chemoattractant across the cell is processed with excitation–adaptation or feedback–

inactivation mechanisms to generate a strong local intracellular signal for pseudopod formation (Iglesias and Devreotes, 2008; Kutscher et al., 2004; Levine et al., 2006). This ‘gradient-directed’ view of chemotaxis might conceal a more general and conceptual insight for oriented movement. A ‘thought experiment’ can be used to substantiate this point. Assume a cell is moving in a very shallow gradient of chemoattractant, which induces a small but significant activation of intracellular signalling pathways in a specific region of the cell. As such, the endogenous spatiotemporal rhythm of pseudopod extensions is subjected to a small gradient-induced spatiotemporal bias that might change the probability of where and when a new pseudopod will form. The thought experiment suggests that the shallow gradient does not induce a pseudopod, but it imposes a positional bias to the pseudopod that is already likely to form. In this ‘pseudopod-directed’ view of chemotaxis, local excitation–adaptation mechanisms are not fundamental for oriented movement. In fact, any mechanism that induces a positional bias of pseudopod formation will induce directed movement, whereas excitation–adaptation mechanisms might improve the accuracy by which a cell can detect shallow gradients.

Navigation in shallow chemotactic gradients

Experiments with *Dictyostelium* have demonstrated that the frequency, growth rate, lifetime and size of pseudopod extensions are not affected by a shallow gradient of chemoattractant (Andrew and Insall, 2007; Bosgraaf and Van Haastert, 2009a). Thus, cell movement continues in a shallow gradient according to the endogenous cycle of self-organising pseudopods. The gradient of chemoattractant has three major effects on pseudopods: selective retraction, oriented extension, and suppression of de novo pseudopod formation (Bosgraaf and Van Haastert, 2009a). Selective retraction is based on the tendency of polarised cells to move with only one pseudopod at a time. Thus, when cells occasionally have several pseudopods, they usually retract all but one pseudopod to retain polarity. In a gradient of chemoattractant, the pseudopod that is best oriented in the direction of the gradient is retained (Andrew and Insall, 2007; Bosgraaf and Van Haastert, 2009a). Although selective retraction is readily observed during chemotaxis, polarised cells usually move with only one pseudopod that is formed by one-way splitting. Such cells chemotax because the gradient of chemoattractant induces a slight bias in the direction of the new pseudopod towards the gradient (Bosgraaf and Van Haastert, 2009a). In buffer, a new splitting pseudopod starts at a short distance from the tip of the current pseudopod, leading to the angle of ~55 degrees that has been observed between splitting pseudopods (Fig. 3). A shallow gradient induces a small bias of the position where a pseudopod starts, such that it is triggered a little closer to the side of the cell that is facing the gradient (Fig. 3). The angle between subsequent pseudopods is not 55 degrees, but is larger or smaller, depending on the location of the chemoattractant. As a consequence of this positional bias, on average, more pseudopods are extended in the direction of the gradient than in other directions.

As discussed above, it is likely that the gradient-induced positional bias of pseudopod extension is the result of combining endogenous pseudopod activators (that would lead to splitting at 55 degrees) and gradient-induced activators. Using *Dictyostelium* signalling mutants, we have demonstrated that at least three pathways contribute to this positional bias: the PI3K pathway, the TorC2 pathway and sGC protein (Bosgraaf and Van Haastert, 2009a). These proteins and/or their products accumulate at the upgradient side of the cell where they increase the probability that

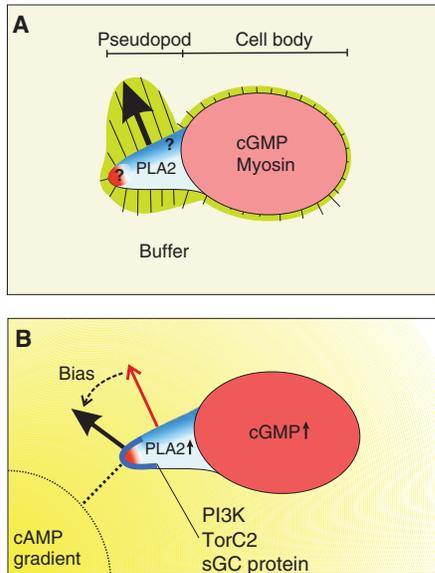


Fig. 3. A pseudopod-centred view of movement and chemotaxis. The figure shows a cell that has formed a pseudopod to the left and will extend a new pseudopod. (A) The effect of intrinsic signals on pseudopod formation in buffer. The lines indicate the measured probability that the new pseudopod will start at a given position. Four regulators have been proposed to be involved in pseudopod extension that occurs in the absence of external cues: cGMP induces myosin filaments that inhibit the formation of de novo pseudopods in the cell body, PLA2 stimulates splitting pseudopods, unknown regulators inhibit the formation of a new pseudopod at the tip of the current pseudopod and unknown regulators also stimulate alternating right–left splitting. The black arrow indicates the most likely direction of new pseudopod extension. (B) The effect of intrinsic and external signals on pseudopod formation during chemotaxis. A cAMP gradient activates cGMP and PLA2, thereby enhancing pseudopod splitting, and locally activates PI3K, TorC2 and sGC protein. The probability that a new pseudopod will form at a given site depends on the relative strength of the endogenous signals and the chemoattractant-induced signals. The red arrow indicates probable direction of a new pseudopod in buffer, whereas the black arrow indicates the direction in a gradient; the change in direction is defined as the bias induced by the gradient. Adapted from a previously published figure (Bosgraaf and Van Haastert, 2009a).

a pseudopod begins to form in that region (Kamimura et al., 2008; Merlot and Firtel, 2003; Parent et al., 1998; Veltman and Van Haastert, 2006) (Fig. 4).

Finally, and in addition to these spatial effects, the gradient also induces suppression of de novo pseudopod formation, by which the persistence time of movement increases. This chemoattractant-enhanced pseudopod splitting is mediated by two other signals through activation of PLA2 and through cGMP production by sGC (Bosgraaf and Van Haastert, 2009a).

The importance of persistence for chemotaxis

Pseudopod splitting induces persistence of movement: the cell has a strong tendency to continue movement in a similar direction (Andrew and Insall, 2007; Bosgraaf and van Haastert, 2009b; Li et al., 2008). As mentioned above, persistence by pseudopod splitting acts as a memory of direction with a time scale of around ten pseudopod extensions. In a gradient, the memory of persistence functions as an integrator of the positional bias that was subjected to the previous ~10 pseudopods. Therefore, the bias that the gradient exerts on each pseudopod can be very small, because the cell

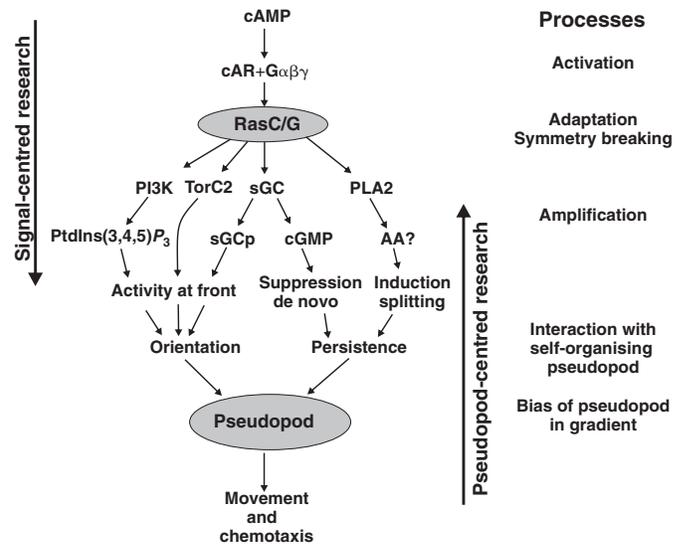


Fig. 4. Integration of signal-centred and pseudopod-centred approaches. Research that investigates and follows the transduction of the cAMP gradient has uncovered the involvement of several signalling pathways, and has demonstrated a central role for Ras activation. Research that investigates how pseudopods are formed has identified the mode of cell movement and the role of the signalling pathways in pseudopod extension. cAR, cAMP receptor; RasC/G, two Ras proteins involved in chemotaxis; sGCp, sGC protein, AA, arachidonic acid as potential mediator of PLA2 activation.

becomes better oriented towards the gradient at each subsequent splitting pseudopod, until a steady state is reached.

The sensitivity of a cell to very shallow gradients depends on the signal-to-noise ratio – i.e. the ability to sense the difference in chemoattractant concentration across the cell (the signal) against the inevitable noise that is produced by stochastic variation of receptor occupancy and activation (Berg and Purcell, 1977; Rappel and Levine, 2008; Ueda and Shibata, 2007; Van Haastert and Postma, 2007). The noise can be reduced considerably if the gradient is measured by the cell repeatedly and then averaged (Ueda and Shibata, 2007; Van Haastert and Postma, 2007). Time-averaging of spatial information requires fast ligand–receptor interactions, combined with spatial and temporal integration of receptor activity. In *Dictyostelium*, the cAMP–receptor complex has a life-time of ~1 second. Slowly diffusing second messengers such as PtdIns(3,4,5)P₃ integrate cAMP–receptor interactions during a ~10 second interval; longer time periods of integration are not possible for these molecules because diffusion causes the loss of spatial information (Van Haastert and Postma, 2007). The memory carried by ten pseudopod splittings extends the integration time to ~180 seconds. Because noise declines with the square root of the integration time, collective averaging of receptor information by slowly diffusing second messengers and pseudopod splitting increases the sensitivity of cells to detect shallow gradients ~13-fold.

Conclusions and future directions

Experiments suggest that the crucial point for cell movement and chemotaxis is the position at the cell surface where a pseudopod is initiated, because, thereafter, self-organisation takes over and the pseudopod grows perpendicular to the cell surface for a specific time and distance. There are probably no fundamental differences

between cell movement in buffer versus movement in shallow or steep chemoattractant gradients. The ordered extension of pseudopod splitting and de novo pseudopod formation that leads to persistent movement in buffer continues with the same frequency in shallow gradients. Low concentrations of gradient-induced signalling molecules are not sufficient to interfere with the timing of the pseudopod cycle, but induce a positional bias where a new pseudopod begins. A steep gradient induces high local concentrations of pseudopod-inducing activity, which not only strongly affects the position of the new pseudopod, but might also interfere with the endogenous timing of pseudopod extensions.

Observations of pseudopod extension in *Dictyostelium* suggest that chemotaxis is not deterministic: the gradient does not determine pseudopod extension, timing or direction. Instead, chemotaxis is probabilistic: cells have a strong basal pseudopod cycle with non-random probabilities for time and place at which pseudopod extension occurs. The gradient of chemoattractant induces a bias of these probabilities such that, on average, more pseudopods are initiated at the side of the cell that is closest to the gradient. This probabilistic view of chemotaxis might also hold true for other models of oriented cell movement, such as electrotaxis or thermotaxis. In electric fields, signalling molecules such as PI3K and sGC translocate to the anode or cathode side of the applied electric field and increase the probability of pseudopod formation, thereby inducing directed movement (Sato et al., 2009; Zhao et al., 2006).

Although much of this discussion is based on the results of studies of *Dictyostelium*, it is very likely that chemotaxis of other organisms is mediated by a similar stochastic bias of a basal pseudopod cycle (Arriuerlou and Meyer, 2005). Pseudopod splitting – the basis of persistent cell movement – has been observed in different cell types (Andrew and Insall, 2007). In the cells of many organisms, chemoattractants regulate F-actin in the leading edge to form protrusions, and regulate myosin filaments in the rear of the cell to suppress the formation of de novo pseudopods and promote the formation of the retracting uropod. However, the molecules that regulate the cytoskeleton and pseudopod extension might vary among different organisms. Specifically, the role of PI3K–PtdIns(3,4,5) P_3 signalling in these processes is probably conserved between *Dictyostelium*, neutrophils and other cell types in other organisms. Conversely, the role of PLA2 is largely unknown in organisms other than *Dictyostelium*, and the function of cGMP in regulating myosin in *Dictyostelium* is mediated by Rho-associated protein kinase (ROCK) in mammalian cells (Li et al., 2005; Schneider and Haugh, 2006; Smirnova and Segall, 2007).

Chemoattractant-induced signalling pathways are often entangled in complex non-linear feedback loops, giving rise to symmetry breaking, adaptation and amplification (Iglesias and Devreotes, 2008). Such complex regulatory pathways are probably incorporated during evolution to improve the signal-to-noise ratio, thereby allowing chemotaxis in more shallow gradients. However, in this stochastic model of pseudopod-driven chemotaxis, none of these loops is essential for gradient sensing. Indeed, *Dictyostelium* mutant cells in which all four signalling enzymes are inhibited (PI3K, TorC2, PLA2 and sGC) show good chemotaxis, but only in very steep gradients (Veltman et al., 2008). In mutant cells, in which only one of the four enzymes is active, chemotaxis occurs in gradients that are ~8-fold shallower, whereas the presence of all four pathways allows chemotaxis in gradients that are ~150-fold shallower (my unpublished results).

I suggest approaching the problem of chemotaxis from two sides (Fig. 4). An approach that follows the signal carried by the cAMP gradient investigates the temporal and spatial activation of signalling pathways. This approach is very powerful for detecting the regulatory mechanisms in the upstream part of the sensory transduction process, such as adaptation and symmetry breaking, which probably occur at the level of Ras activation (Zhang et al., 2008). Conversely, a pseudopod-centred approach can provide more mechanistic insight into how the signalling pathways synergise, how they interact with the cytoskeleton and how they influence pseudopod formation. These methods will allow characterisation of the basal mode of pseudopod formation, which will be instrumental for our understanding of chemotaxis.

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