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Navigating in tissue mazes: chemoattractant interpretation in complex environments

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Guided cell movement is essential for development and integrity of animals and crucially involved in cellular immune responses. Leukocytes are professional migratory cells that can navigate through most types of tissues and sense a wide range of directional cues. The responses of these cells to attractants have been mainly explored in tissue culture settings. How leukocytes make directional decisions *in situ*, within the challenging environment of a tissue maze, is less understood. Here we review recent advances in how leukocytes sense chemical cues in complex tissue settings and make links with paradigms of directed migration in development and *Dictyostelium discoideum* amoebae.

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Introduction

Many types of cells are guided by attractive or repulsive environmental cues. Unicellular organisms migrate to find resources or avoid predators. In multi-cellular organisms, directed cell migration underlies development, regeneration and immune control. The migratory capacity of leukocytes is particularly admirable as these cells do not have an organ of residence but routinely patrol the organism for signs of infection or damage. To do so they crawl along and traverse blood vessels and navigate through almost all types of tissues. All this is achieved with remarkable efficiency, adaptability and precision that would be fortuitous without powerful mechanisms to interpret external guidance cues. Here we discuss recent *in situ* evidence of leukocyte responses to chemoattractants in relation to paradigms from chemotaxis of *Dictyostelium discoideum* amoebae or developmental migration processes.

In situ evidence for interstitial gradients

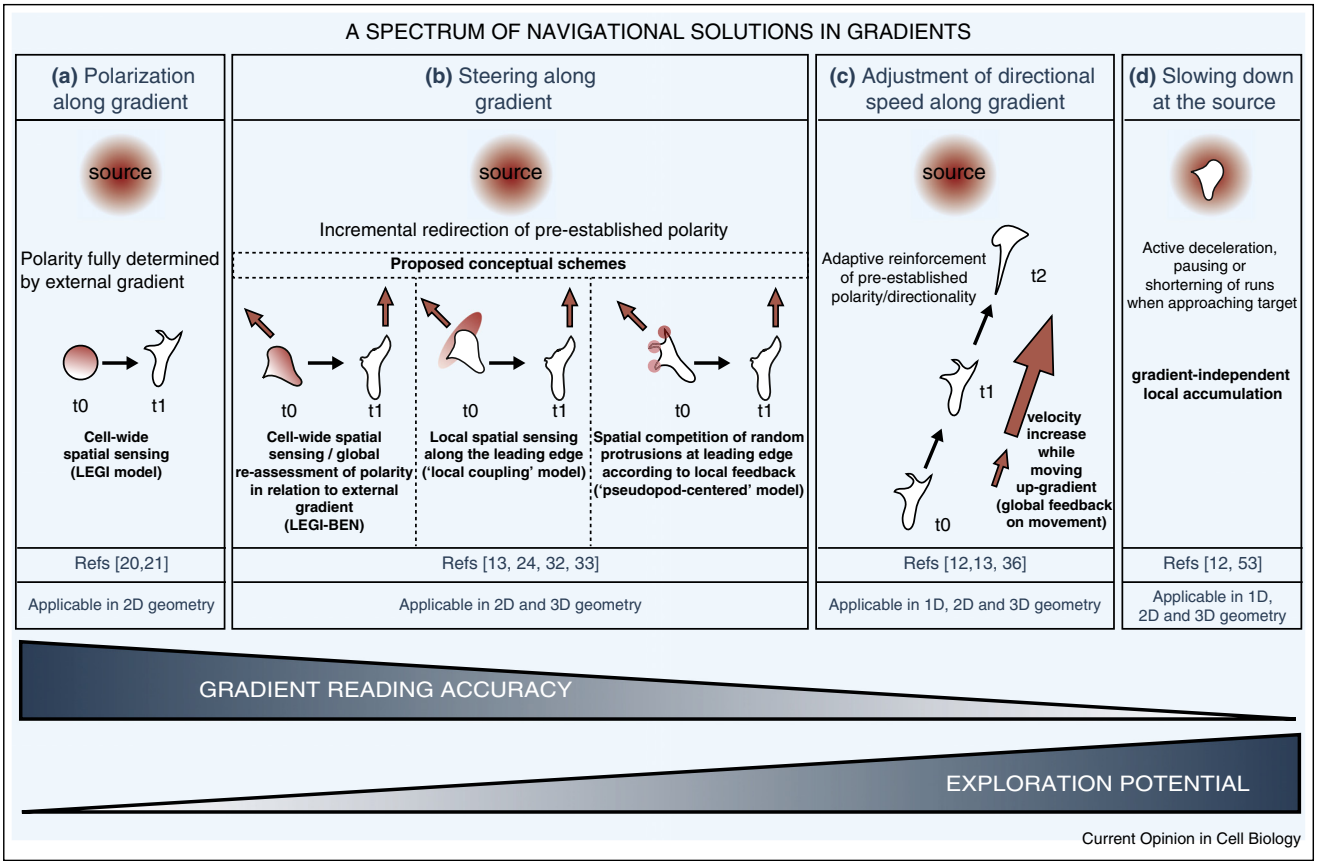
Much has been learned about how leukocytes exit the blood stream to enter target tissues, a process highly regulated by chemokines as reviewed elsewhere [1–4]. Once in the target tissue, leukocytes are further guided by chemokines and other attractants. For a while this was assumed to occur through 'chemotaxis', the directed migration along concentration gradients of diffusing attractants. However, the concept of gradient-driven interstitial migration was challenged when new methodology for deep tissue imaging revealed a surprisingly high degree of random leukocyte motility *in situ* [5,6]. While this argued for substantial "non-tactic" contributions like anomalous diffusion [5,7], regulation of motility levels [7,8] and contact guidance via tissue geometry [5] several new studies have now provided solid evidence for the existence of functional gradients *in vivo*. Gradients of H_2O_2 were shown to recruit zebrafish neutrophils to sites of wounding [9,10]. Interstitial chemokine gradients in lymph nodes were associated with directed migration of B cells [11] and chemokine gradients whose guidance function depends on binding to extracellular heparan sulfate (HS) proteoglycans were shown to attract zebrafish neutrophils to sites of bacterial infection [12••]. Similar 'haptotactic' chemokine gradients were shown to directionally guide dendritic cells to lymphatic vessels in mouse skin tissue [13••]. Finally, Ulvmar *et al.* demonstrated that functional chemokine gradients in mouse lymph nodes can be established through ligand sequestration by atypical chemokine receptors [14••], a mechanism previously demonstrated in developmental migration of primordial germ cells [15••] and the lateral line primordium in zebrafish [16••,17]. Thus, in some physiological settings gradients are instructive for leukocyte migration and mechanisms such as extracellular matrix binding or sequestration by scavenger receptors were shown to establish and maintain these gradients.

Principles of spatiotemporal information processing

Several models of eukaryotic chemotaxis have been proposed on the basis of *in vitro* studies with neutrophils and *D. discoideum* amoebae. A general premise is that eukaryotic chemotaxis is different from prokaryotic chemotaxis. Most bacteria use a fast swimming mode of locomotion and employ flagella, which in gradients change rotation pattern such that decreasing concentrations trigger directional changes while increasing concentrations favor persistence [18]. This temporal sensing strategy allows interpretation of differences in attractant concentration

that are not discernible along the small detection surface of bacteria. Animal cells are big and comparably slow and their locomotion is mediated by the cytoskeleton. A key element in this process is the acquisition of cytoskeletal polarity with protrusive extensions at the leading edge and a retracting zone at the rear [19]. The orientation of this front-back polarity towards a gradient has been proposed to result from spatial information processing, whereby differential signal input across the cell generates internally amplified gradients of messenger cascades, which then direct polarity (Figure 1a). Even when their cytoskeleton is disrupted, cells can form internal gradients, demonstrating that spatial signaling is at least partially independent of the locomotive response [20,21]. The amplification of a shallow external gradient into an explicit polarity axis was explained by a local excitation and global inhibition (LEGI) process [22], whereby a local signal triggers actin-polymerisation at the cell front and a global inhibitor, which may be of biochemical [22] or mechanical [23] nature, prevents this from occurring at

Figure 1



A spectrum of navigational solutions in gradients. The various responses observed in gradients (polarisation along gradient, steering, adjustment of directional speed and slowing down at the source) can be seen as navigational solutions with a different trade-off between gradient reading accuracy and exploratory potential and of differential applicability in 1D, 2D and 3D matrix geometry. Black arrows represent transitions in time and red arrows represent cell movement. **(a)** Polarisation along gradients. With no prior internal asymmetry (minimum exploration potential) and a strong external asymmetry (maximum gradient reading accuracy), polarity can be fully determined by external gradients, through cell-wide spatial sensing. This is applicable in 2D settings with no other polarising signal present (in confining 1D channels or 3D matrices leukocytes self-polarise independently of external gradients). **(b)** Steering along gradients. In pre-polarised cells sensitivity of detection is highest at the leading edge and this polarised state can be resistant to reversal (i.e. reversal requires a steep gradient in the opposite direction). Instead, global reassessment of internal polarity in relation to the external gradient ('LEGI-BEN' model) or local spatial sensing along the leading edge ('local coupling' model) can trigger biased extension of protrusions at the front and incremental re-orientation. Alternatively, cells may extend protrusions in an uninformed, random manner, which compete along the leading edge according to differential positive feedback, leading to incremental steering ('pseudopod-centered' model). This strategy ensures high exploration activity, through self-forming protrusions, and high gradient reading accuracy, through redirection of polarity, but requires a 2D or 3D field of exploration. **(c)** Adjustment of directional speed as cells move along a gradient. This represents a navigational solution whereby polarity is not redirected but reinforced in an informed manner. Here the gradient reading accuracy is lower than in a and b but the exploratory potential is higher, as the cells explore more space through random advancement. In addition this strategy is suitable for migration in 1D tracks as well as 2D and 3D fields. **(d)** At the end of the spectrum, local deceleration/dwelling ensures that cells that fail to read a gradient and randomly arrive at the target can also locally accumulate. This is the least demanding strategy for the cells as it makes full use of random exploration of the environment and has no special requirements in terms of tissue geometry or gradient shape.

the rear. A more evolved version of this model (LEGI-Biased Excitable Network or LEGI-BEN) incorporates an internal stochasticity in this process to account for observed biased-random walk behaviours of cells [24••,25,26]. Here, actin polymerisation behaves as an excitable network on which a LEGI mechanism acts to transiently reduce the excitation threshold [24••,25,26] (Figure 1b).

Establishment of a polarity axis often arises independently of external gradients, e.g. in response to uniform attractant, as a result of mechanical confinement or spontaneously (possibly by stochastic amplification of internal fluctuations) [27]. In such cases any additional attractant gradient acting ''on top'' needs to redirect the orientation of the polarity axis, meaning that polarity and directional sensing are not necessarily outcomes of the same process. In pre-polarised cells the sensitivity to attractant is usually asymmetric, with the leading edge being more responsive. This was shown in classical micropipette experiments, where placing attractant at the uropod was more likely to trigger a U-turn than reversal of polarity [22,28]. Differential sensitivity was also apparent in optogenetic experiments in zebrafish, where photoactivation of Rac more readily steered neutrophils when applied along the leading edge of already polarised cells [29••]. Polarised sensitivity can be developmentally determined; for example Dictyostelium amoebae are more resistant to changing direction and prone to perform U-turns at late developmental stages [30]. Interestingly, using a cocktail of inhibitors to block actin dynamics but preserve cytoskeletal structures, it was shown that polarised sensitivity depends on cytoskeletal architecture but not on cytoskeletal dynamics [31••]. Thus the cytoskeleton appears to primarily provide structural support for polarised responses to stimuli, rather than contribute through intracellular transport, force generation or cell deformation [31••].

As a conceptual framework for guidance of pre-polarised cells a 'local coupling' model was proposed which suggests that spatial sensing across the leading edge, rather than the entire cell, can drive biased protrusion extensions leading to small turns and incremental redirection of polarity (Figure 1b) [32••]. Here, self-polarisation randomises cell orientation but local coupling of the internal and externally triggered signal networks at the leading edge leads to small turns towards the stimulus. This model dissociated global cell polarity from local protrusion dynamics and proposed that information processing happens at the leading edge (Figure 1b). This is a subtle distinction from models that propose global coupling/integration between a cytoskeletal oscillatory network (CON) and a signal transduction excitable network (STEN) at the level of the whole cell [24••,26] (Figure 1b). The CON-STED models take into account a signal-induced long-range inhibitor (i.e. a LEGI process) as a means of integrating signal inputs across the cell, i.e. for every signalling event at the front of the cell there is a corresponding event at the opposite end of the cell.

Signal interpretation can be even less deterministic in weak gradients. According to statistical analyses of protrusion dynamics in *D. discoideum*, spatial resolution of the gradient before protrusion extension is not an absolute requirement. Instead, direction may be determined by autonomous protrusions that are generated by splitting of existing pseudopods and are selectively stabilised when encountering increasing ligand concentrations (Figure 1b) [33–35]. This is consistent with the idea of 'pseudo-spatial'/'pseudo-temporal' sensing, discussed in the 1980s [36], whereby randomly extending protrusions spatially compete along the leading edge and every single protrusion integrates signal intensity over time. The protrusion experiencing the steepest temporal increase dominates and then reorients the cell. This so-called 'pseudopod-centered' model, much like the 'local coupling' model and unlike LEGI-based models, places directional decision-making at the level of protrusions/leading edge while polarity merely responds secondarily to the instructions of the dominant protrusion/part of the leading edge (Figure 1b). A question evoked by this conceptual scheme is how local feedbacks at the level of protrusions are ultimately transformed into global effects on cell polarity. One possibility is that local protrusions and global polarity represent distinct networks acting at different scales, whereby outputs from protrusion networks provide an average, global feedback to the polarity network. Multiple small protrusions would cause weak feedbacks in opposing directions and thus be ineffective. On the contrary, when a protrusion dominates sufficiently over others, the global feedback would be strong enough to perturb the polarity network and redirect the cell. The idea of global feedback from protrusions to polarity is supported by *in vivo* evidence in zebrafish neutrophils [29••]. Here, PI3 kinase was found to be essential for both protrusions and polarity. In wild type cells, local photoactivation of the small GTPase Rac at parts of the leading edge could fully redirect the cell. By contrast, in PI3K-inhibited cells photoactivation of Rac could rescue protrusion defects but not global polarity defects, suggesting that protrusions and polarity are not manifestations of one and the same signaling network and that full redirection of the cell requires feedback from protrusions to polarity.

In line with the concept of global feedback and despite the prevailing dogma of spatial sensing, eukaryotic cells show signs of temporal memory. This was described in early experiments where Dictyostelium amoebae [37] and neutrophils [38] responded to temporal rises in uniform attractant with directionally persistent motility, while temporal decreases led to directional changes. Moreover, early trajectory analyses of Dictyostelium in spatial gradients showed two behavioural responses: first,

correcting cell path by steering in the direction of the source and second, moving faster up-gradient than down-gradient [36] (Figure 1b and c). The latter response (often referred to as orthotaxis [6,12••,39]) was proposed to arise from global temporal feedbacks on cell speed, as cells move through the gradient. Recently, using microfluidic setups to mimic travelling waves of attractant, *Dictyostelium* amoebae were shown to maintain directional movement when re-stimulated within a limiting period of 6 min and this cellular memory was proposed to underlie self-organised aggregation behaviours in response to pulsatile attractant [40].

Taken together, there is general agreement that polarity and directional sensing are different processes, whereby polarity and protrusions are manifestations of an internal motility network that can be redirected by external gradients. There are some differences across chemotaxis models as to first, whether this happens through a coupling/integration of internal and external networks (see 'LEGI-BEN/CON-STED' and 'local coupling' model) or through local/global feedback loops (see 'pseudopod-centered' model), second, whether the directional sensing unit is the entire cell (LEGI-BEN/CON-STED), the leading edge ('local coupling' model) or the individual protrusions ('pseudopod-centered' model). In light of these discrepancies, the conceptual categorisation of bacterial and eukaryotic chemotaxis into 'temporal' *versus* 'spatial' strategies appears more ambiguous than originally proposed.

Leukocyte behaviour in interstitial gradients *in situ*

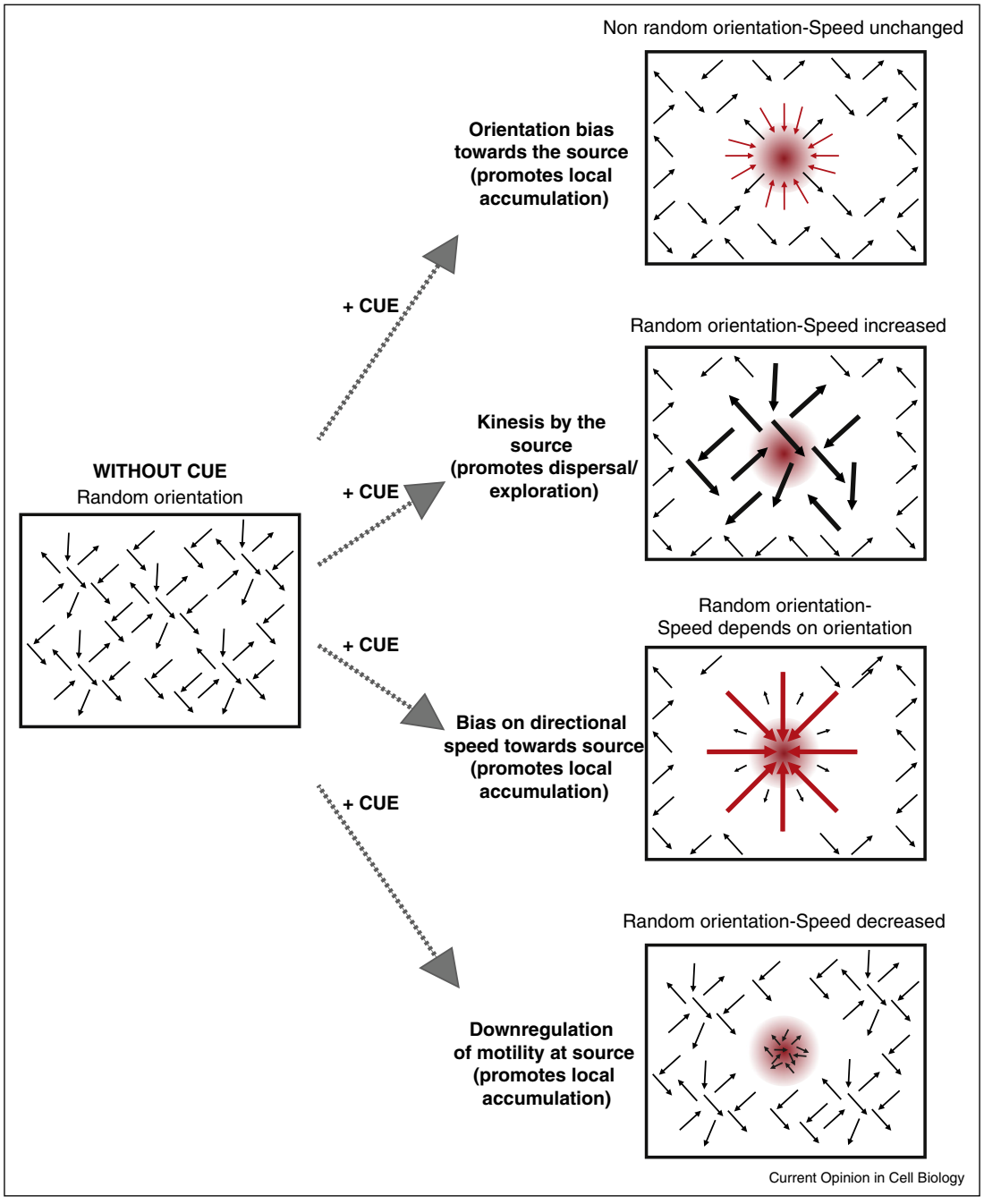
In situ evidence for sub-cellular information processing in leukocyte interstitial navigation is scarce, but some insights have been deduced from cell trajectory analyses. Initial studies revealed kinetic effects of attractants in tissue (Figure 2). For example chemokines increase random T cell motility within lymph nodes, thereby promoting the detection of rare antigen presenting cells [8]. Similarly, chemokines were found to enhance T cell speed, facilitating detection of rare *Toxoplasma gondii* parasites during infection [7]. Analysis of leukocytes migrating in interstitial gradients [12••,13••] revealed patterns analogous to Dictyostelium. Two types of directional biases were detected. The first was a bias in average orientation (Figure 2) as reported for dendritic cells migrating along gradients of Ccl21 in the skin [13••]. Such effects at the level of a cell population may either reflect active turning or prolonged directional persistence. The second was a bias on directional speed, favouring fast movement up-gradient (Figures 1c and 2). While this component was again detectable in dendritic cells migrating along Ccl21 gradients [13••] it was even the predominant effect observed in zebrafish neutrophils moving along Cxcl8 gradients [12••]. Here, orientation of movement per se was not affected [12••]. Thus, cells can apparently chemotax merely by adjusting directional speed without necessarily redirecting polarity. This gradient response could explain earlier reports of chemokine-dependent 'jumps' of T cells towards antigen-presenting cells [41] and directional acceleration of positively selected thymocytes towards the chemoattractive thymic medulla [42]. Teleologically, adjustment of directional speed through global feedback, seems like a useful strategy in at least two situations: first, when the gradient is too noisy/discontinuous to be spatially resolved across the leading edge or the entire cell (i.e. the external spatial asymmetry is not strong enough to redirect polarity) and second, when movement is physically constrained to one-dimensional tunnels/tracks, such as tissue interstices or scaffolds [5], and cells have limited geometrical freedom to turn (Figure 1c). It will be important to know what the determinants of directional speed at the sub-cellular level are and how these are influenced by gradients. Actin flow is an interesting candidate; it is perhaps the most universal determinant of cell speed in confined environments [19,43], it has directionality, may be influenced by attractants through effects on actin-polymerisation and can provide a secondary positive feedback on cell polarity and persistence [44].

Leukocytes can also slow down at target sources of attractant, providing an additional means of cell positioning (Figures 1d and 2). This effect was described for zebrafish neutrophils upon arriving at sources of Cxcl8 at sites of infection or wounds [12••,45] and for mouse T cells coming into contact with antigen presenting [46] or virus-infected cells [47]. Local deceleration was shown not to be gradient-dependent per se, as inhibition of chemokine-HS interaction, which led to loss of stable gradient formation and directionality, still preserved a certain degree of deceleration and accumulation at the source [12••]. Thus, chemokine-triggered 'breaks' on leukocyte movement can be seen as the last resort, whereby cells that fail to read the gradient and randomly find the source can still locally accumulate (Figures 1d and 2).

Strategies to enhance information sampling, resolution and sensitivity of detection

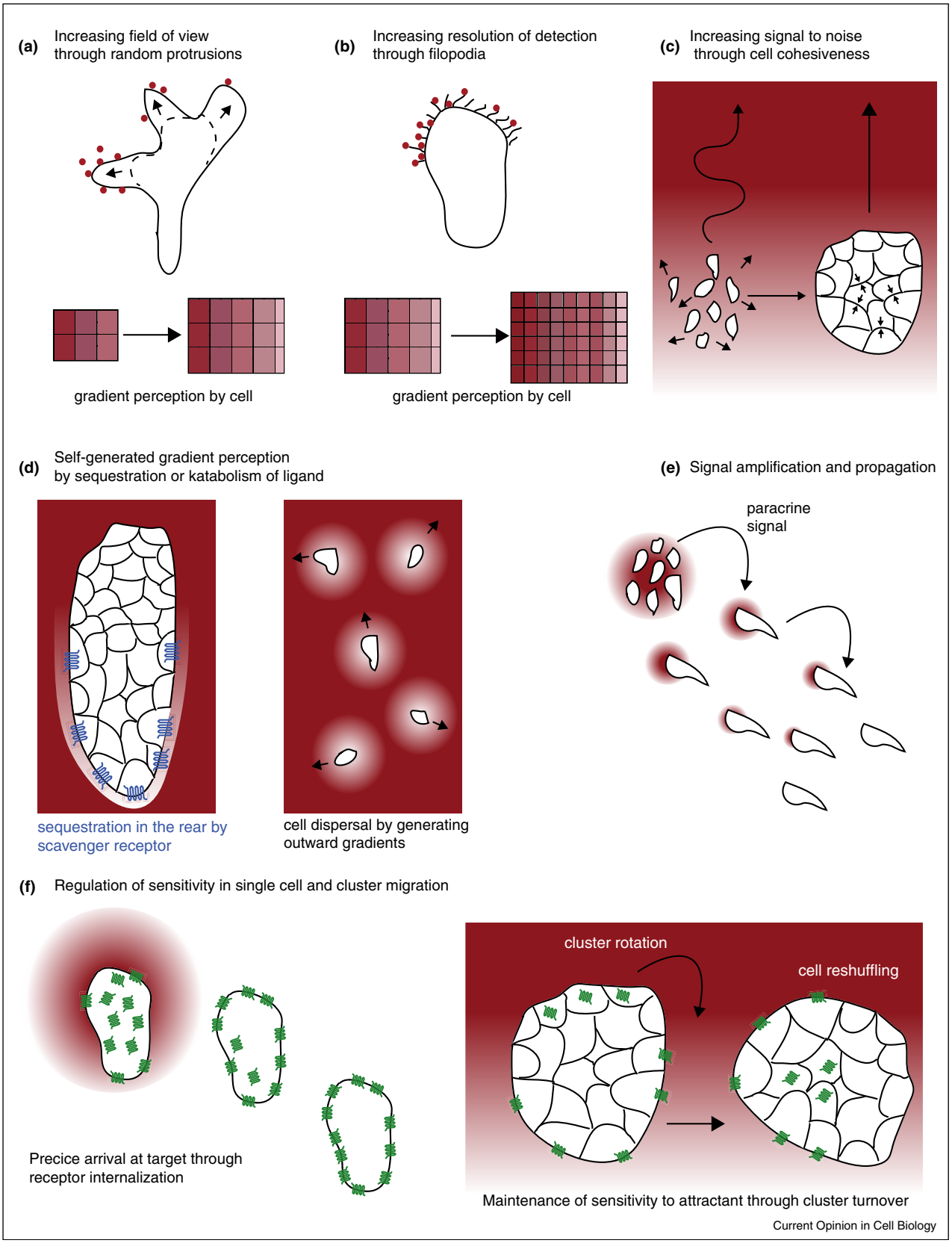
For an ideal response to gradients cells should first, maximise their spatiotemporal sampling-range, second, optimise the resolution of signal and third, precisely adjust their sensitivity to the ambient levels of attractant. Generation of random exploratory protrusions, as described in *D. discoideum*, can be seen as a form of information sampling (Figure 3a). Such active protrusions are evident in leukocytes *in vivo*; they range from long filopodia in macrophages, to extensive veils in dendritic cells or the smaller lamellipodia of neutrophils and lymphocytes. Another way how cells increase their 'field of view' is the use of temporal memory that was shown to underlie perception of travelling waves of attractant in

Figure 2



Leukocyte trajectory modulations by chemoattractants observed *in vivo*. Rectangles represent migration fields and arrows represent steps of movements (trajectory units). The state of the vectors is represented in the absence (left panel) *versus* in the presence of the cue (right panels, pink source in the centre). Before addition of the cue the distribution vectors is isotropic in terms of orientation and speed. **(a)** A bias in vector orientation is found when cells at a given time and distance from the source are more likely to move towards (red vectors) than away from the source. **(b)** Kinetic effects are found when average vector size is larger regardless of orientation relative to the source. This effect does not lead to local accumulation but favours exploration and dispersal. **(c)** A bias on directional speed is evident when vector size positively correlates with orientation towards the source, i.e. addition of the cue leads to larger vectors towards the source (red) and smaller vectors in the opposite direction. **(d)** Down-regulation of motility at the source is reflected by a decrease of average vector size at the target zone independently of orientation.

Figure 3


(a) Increasing field of view through random protrusions
gradient perception by cell
(b) Increasing resolution of detection through filopodia
gradient perception by cell
(c) Increasing signal to noise through cell cohesiveness
(d) Self-generated gradient perception by sequestration or katabolism of ligand
sequestration in the rear by scavenger receptor
cell dispersal by generating outward gradients
(e) Signal amplification and propagation
paracrine signal
(f) Regulation of sensitivity in single cell and cluster migration
Precice arrival at target through receptor internalization
cluster rotation
cell reshuffling
Maintenance of sensitivity to attractant through cluster turnover
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D. discoideum. In a spatial gradient, this extends the exploration window beyond the scanning range of protrusions.

Studies of primordial germ cell migration in zebrafish have revealed examples of how cells may increase signal resolution and adapt their sensitivity *in situ*. Primordial germ cells are directed by gradients of Cxcl12 to the developing gonad, by biased formation of blebs at the front of the cell [48] and polarised distribution of internal pH [49]. High-resolution imaging revealed extensive filopodia along the cell front that internalised Cxcl12 [50••]. Perturbation of filopodia formation compromised orientation in the gradient, indicating their sensory function. Here, in analogy to the scanning of a digital image, sensory filopodia increase the field of view, while they might also enhance the resolution of the image (i.e. the detection of small differences in attractant concentration) by increasing local receptor density (Figure 3b). Filopodia were also found to improve the accuracy of guidance in zebrafish neural crest cells [51]. It will be interesting to see whether filopodia observed in leukocytes [52] have a contribution in gradient sensing. With regards to adaptation, receptor internalisation was found to play a key role in gradient interpretation by primordial germ cells (Figure 3f) [53••]. When Cxcl12 receptor internalisation was blocked through C-terminal truncation, cells were found to often overshoot the target. This suggests that receptor internalisation can fine-tune and actively restrict excess motility in proximity to the source, by shortening the 'run' phases and allowing more frequent trajectory corrections. This has interesting parallels with the deceleration effects of chemokines on leukocyte migration, discussed above. Along these lines, the genetic loss of one of the G protein coupled receptor regulatory RGS proteins, which are responsible for appropriate chemokine receptor signal adaptation, led to defective localisation of mouse neutrophils at sites of infection [54]. The dynamics of chemoattractant receptors in leukocytes remain to be assessed *in situ*, although *in vitro* evidence has suggested roles for receptor internalisation, oligomerisation and redistribution in gradients [55–57]. Theoretical modelling has also suggested that uneven receptor positioning along the cell surface, whether pre-patterned or induced by chemoattractants, may affect gradient resolution capacity and compensate for any undesirable biases due to the assymmetrical shape of the cell [58].

Higher order information management during collective and self-organised cell behaviours

Information management can be strikingly different when cells are migrating collectively. An interesting paradigm was described during chemotaxis of clusters of malignant B cells [59•] and neural crest cells [60]. In both scenarios chemotaxis along chemokine gradients was more accurate when cells migrated in clusters compared to individually migrating cells. Theoretical modelling suggests this property can emerge by cancelling out noise across the cell collective [59•]. While all cells within the migrating collective seem to sense the attractant, forces generated in random directions are averaged out because of cell cohesiveness and contact inhibition of locomotion, thus minimally affecting the meandering index (Figure 3c) [59•,60]. Moreover, in contrast to single malignant cells, which are susceptible to receptor endocytosis-driven chemorepulsion/desensitisation *in vitro*, clusters maintain sensitivity to chemokine at high concentration through cluster rotation and leader cell turnover [59•] (Figure 3f).

Another emerging paradigm is the autonomous generation of gradients by migrating cells (Figure 3d). During lateral line primordium migration in zebrafish, directed migration occurs across a stretch of uniform attractant [16••,17]. Here, the directional perception is generated by the primordium itself through asymmetric modification of the attractant field, by chemokine sequestration at the rear of the collective. It is unclear whether such a mechanism may also occur in leukocyte migration, but worth considering as atypical chemokine receptors have been reported to have cell-autonomous effects in these cells [61]. Interestingly, autonomous gradient generation at the single cell level was recently described during melanoma cell dispersal [62••]. Here individual melanoma cells act as sinks of their own attractant by breaking down lysophosphatidic acid (LPA), an attractant locally present in malignant tissue, thereby generating outward gradients of LPA and promoting cell spreading (Figure 3d).

Neutrophil swarming is yet another example of collective migration that has been observed in inflammatory situations in mouse and zebrafish tissue [63,64••,65], and bears striking similarities with the aggregation of *D. discoideum* amoebae. *In vitro* studies with human neutrophils [66••] and mouse genetic experiments [64••] have determined this response to be highly dependent on neutrophil

(Figure 3 Legend) Strategies to improve or self-generate interpretation of chemoattractants in single cells or collectives. **(a)** Extension of exploratory protrusions increases the field of exploration. Chemoattractant molecules are shown in red. **(b)** In primordial germ cells, filopodia increase the units of detection (receptor-bearing membrane), improving the resolution of the gradient. **(c)** Increasing signal to noise ratio by averaging information across a cell collective. Forces in random directions are cancelled out through cohesiveness and contact inhibition of locomotion. **(d)** Self-driven directional perception. —In the lateral line primordium gradient perception is generated through polarised sequestration of ligand by scavenger receptors at the rear of the collective. Melanoma cells catabolise attractant along a uniform field of ligand, leading to outward gradients and cell dispersal. **(e)** Signal relay and amplification through autocrine and paracrine effects. **(f)** Management of sensitivity in single cells and collectives. Primordial germ cell motility is down-regulated at the source via receptor internalisation, allowing precise arrival. Clusters avoid chemorepulsion by cluster rotation and renewal of cells with available receptors at the surface of the collective.

production of their own attractant, leukotriene B4 (LTB4). Here paracrine LTB4 signalling is thought to extend neutrophil recruitment range, while autocrine LTB4 signalling may further enhance directionality (Figure 3e) [66••].

Concluding remarks

Guidance of cells is demanding and cannot afford to fail. The spectrum of strategies to read gradients or navigate independently of gradients can be seen as evidence of robustness as well as adaptation to specific physiological contexts. For example gradient interpretation in some situations may be geared towards accuracy whereas in others it may be optimised for better exploration (Figure 1). We are only beginning to understand interstitial guidance in its real complexity and dimensions. Recent advances in imaging technologies, breakthroughs in gene targeting, the implementation of optogenetics to spatiotemporally manipulate cell signalling [67,68] and accessible model organisms provide an ideal ground for further *in situ* interrogations of cell guidance and new answers to old questions.

Acknowledgements

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- •• of outstanding interest

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